

**VIETNAMESE LOCAL CHICKEN BREEDS: GENETIC DIVERSITY AND
PRIORITISING BREEDS FOR CONSERVATION**

Dissertation to obtain the PhD degree
in the international PhD program for Agricultural Sciences in Göttingen (IPAG)
at the Faculty of Agricultural Sciences,
Georg-August Universität, Göttingen, Germany

Presented by

Ngo Thi Kim Cuc
(born in Hanam, Vietnam)

Göttingen, April 2010

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1. Referee:

Prof. Dr. Henner Simianer
Animal Breeding and Genetics Group
Department of Animal Sciences
Georg-August Universität, Göttingen

2. Co-referee:

Prof. Dr. Eva Schlecht
Animal Husbandry in the Tropics and Subtropics Group
Universität Kassel and Georg-August Universität Göttingen

In collaboration with

Dr. Steffen Weigend
Institute of Farm Animal Genetics
Friedrich- Loeffler-Institut (FLI)
Neustadt-Mariensee

ACKNOWLEDGEMENTS

I would like to greatly acknowledge:

Prof. Dr. Henner Simianer for offering me the opportunity to work on this interesting topic and for accepting this thesis as well as for providing me fruitful ideas to develop the whole study.

Prof Dr. Eva Schlecht for taking over co-referee and for accepting this thesis.

Dr. Steffen Weigend for welcoming me to his laboratory and for valuable discussion. He always brought the best solutions for difficult situations in the laboratory works.

Prof. Dr. Clemens B.A. Wollny, Prof. Dr. Hoang Van Tieu and Dr. Vu Chi Cuong for initiating this project.

Mrs. Annet Weigend and Ms. Anke Flörke for their technical assistance with the laboratory works in Mariensee.

Dr. Linn Fenna Groeneveld and Dr. Herwin Eding for giving me useful suggestion and discussion.

Prof. Dr. Eildert Groeneveld and his working group for offering me a warm reception.

Mrs. Ute Döring for giving me help any time I needed it.

Cong, Tamina and Farai for giving interesting talks and exchanging experiences.

Bohani Mtileni for offering me his kindness to correct English whenever I needed.

My colleagues in the Animal Breeding and Genetics Group for help, warm working environment.

Vietnamese farmers for providing chicken blood samples and useful information on their chickens.

Vietnamese local conservationists for supporting during my field work.

German Academic Exchange Service (DAAD) and German Federal Ministry of Education and Research (BMBF) for the financial supports.

Vietnamese student association in Göttingen for making my stay in Göttingen enjoyable.

My parents, my aunt, my parent in-law for taking care of my daughter and for their encouragement, moral support, tolerance, inspiration during my study.

My husband Le Van Hai and my daughter Le Huong Ly for their great patience and sympathy during the time I was away from home. Without their supports, I can not finish this study. Thank you a lot for all.

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SUMMARY

The overall goal of this study was to characterize the genetic diversity of the Vietnamese local chicken breeds and to identify population priorities for conservation. The specific aims were 1) to assess and explain the population genetic structure of the Vietnamese breeds, 2) to characterize the Vietnamese breeds in relation to the Chinese breeds and wild chickens, 3) to estimate conservation potentials for conservation priorities of the Vietnamese breeds, and 4) to define an optimal allocation of limited conservation funds to them.

First genetic diversity within and between the Vietnamese breeds was analysed at the autosomal level using 29 microsatellites. A total of 353 individuals of nine Vietnamese local breeds and two breeds of Chinese origin were studied. The Vietnamese breeds were sampled from the northern and southern parts of Vietnam while the two Chinese breeds (NIAS Chinese breeds) have been kept at the National Institute of Animal Sciences, Hanoi. The results revealed a high level of diversity within Vietnamese breeds. Analysing the genetic structure using the software package STRUCTURE suggested an optimal clustering at $K = 6$. These groups encompassed four homogeneous clusters, one formed by the two Chinese breeds and the other three representing a single breed each: the Mekong Delta breed Ac, the South Central Coast breed Choi, and the Red River Delta breed Dong Tao. The six remaining breeds formed two additional admixed clusters. This finding indicates that sub-structuring of the Vietnamese chicken breeds is related to their geographical distribution. The two NIAS Chinese breeds are genetically distinct from the Vietnamese breeds.

Mitochondrial DNA D-loop sequences were used to determine the degree of shared mtDNA haplotypes between the studied breeds. A 455-bp fragment of the mtDNA D-loop region was sequenced in 222 chickens of nine Vietnamese breeds and two NIAS Chinese breeds. As reference, a skeleton was created based on chicken mtDNA sequences taken from the Genbank which assigned clades to suggested regions of domestication in chickens. Haplotypes of the nine Vietnamese and two NIAS Chinese breeds were aligned together with these sequences. The mtDNA haplotypes formed eight clades. The majority of individuals of the two NIAS Chinese breeds grouped together in one clade. Although the Vietnamese breeds were distributed across all eight clades, most of them clustered in

three main clades originating from from Yunnan, South and Southwest China and/or surrounding regions.

The differentiation of Vietnamese breeds from Chinese chickens was characterised. For this, as reference, data of 14 indigenous breeds from China (Reference Chinese breeds) and three Red Jungle Fowl populations (*Gallus gallus gallus* and *Gallus gallus spadiceus*) genotyped at the same microsatellite loci in a previous study were included. Analysing the genetic structure indicated that the Vietnamese breeds formed a homogeneous group separated from the other breeds (except for the Chinese Chahua breed). This finding provides additional evidence that the Vietnamese gene pool is genetically different from the Chinese gene pool even when a wider range of breeds is considered. Another analysis was also used to find the degree of uniqueness of the Vietnamese breeds relative to a set of three Red Jungle fowl populations. Compared to the reference Chinese breeds, the contribution of the Vietnamese breeds to the set of Red Jungle Fowl populations is lower. This suggests that the Vietnamese breeds are more closely related to the Red Jungle fowl populations than the reference Chinese breeds.

The second objective of this thesis was to estimate the conservation potential of the Vietnamese breeds and to derive optimal allocation of conservation funds to maximize genetic diversity conserved between these breeds. The Weitzman approach for assessing alternative conservation strategies for genetic diversity between breeds was used. The approach combines genetic diversity assessed at the molecular level and extinction probabilities estimated by socio-economic factors to derive conservation priorities of breeds based on their conservation potential. To investigate an optimal allocation of conservation funds, three different models were applied. These models reflect the range of possible functions between costs and effects in typical conservation situations. Under the assumptions made it is expected that half of the genetic diversity of the Vietnamese breeds will go lost within the next 30–50 years if no conservation efforts are taken. Conservation potential of the Vietnamese chicken breeds varies considerably. The optimum conservation strategy to maximise genetic diversity between the Vietnamese breeds should prioritize the breeds with the highest conservation potential. Population priorities for allocation of conservation funds to the Vietnamese breeds do not depend on which cost model was used. The three local breeds Te, Dong Tao and Ac have the highest conservation potential and should be the prime candidates for conservation programs.

1st CHAPTER

General introduction

Background

Vietnam is an agricultural country with 70% population living in rural area. More than 80% of the total agricultural households keep chickens (Vang, 2003 and Burgos *et al.*, 2008). In 2006, the chicken population in Vietnam was estimated about 152.7 millions. The distribution ranges from 2.9 million in the Northwest to 40.6 million birds in the Red River Delta. Local chickens make up more than 70% of the country's total chicken population (Desvaux *et al.*, 2008). They are mainly kept in the traditional extensive backyard/household production, representing about 94% of all poultry producers (Hong Hanh *et al.*, 2007).

Chicken is the country's second most important meat source after pork (Burgos *et al.*, 2008) and plays an integral role in the smallholder farming systems. They are used to meet the multiple social, economic and cultural needs of households (Epprecht, 2005 and Burgos *et al.*, 2008). Vietnamese local chicken breeds are specific for particular regions and they are assumed showing specific adaptation to climate, disease, local low input and low output production system (Vang, 2003). Hence they may represent a large natural gene pool as reservoir for future breeding to meet specific objectives.

Vietnamese local chickens consist of different phenotypes kept in distinct agro-ecological zones of Vietnam, which stretches 1 600 km from North to South. Geographical isolation of the populations could result in sub-structuring through drift, mutation and divergent selective forces. However, it is not known to what degree Vietnamese local chicken populations differ. An assessment of genetic diversity using molecular markers may serve as a initial guide to identify unique and valuable genetic resources. Recently, several studies to assess genetic structure of chicken populations using molecular tools such as microsatellite markers (Hillel *et al.*, 2007; Muchadeyi *et al.*, 2007; Chen *et al.*, 2008; Berthouly *et al.*, 2008; Granevitze *et al.*, 2007; 2009 and Bodzsar *et al.*, 2009) and mitochondrial DNA (Liu *et al.*, 2004; Liu *et al.*, 2006; Oka *et al.*, 2007; Muchadeyi *et al.*, 2008 and Silva *et al.*, 2008) were published. An assessment of the genetic structure employing these molecular tools provides different insights into diversity within and between indigenous chicken populations. These two marker types have a different mode of inheritance. Microsatellites are autosomal markers while mitochondrial DNA is maternally inherited.

The genetic potential of indigenous chickens as a reservoir of genomic variation and major genes with relevance to improve adaptability has already been reported by several reports (Horst and Mathur, 1992; Horst, 1989; Garces and Casey, 2003 and FAO, 2007a). Understanding about the existing variation that already exists and how it can be conserved and accessed effectively needs to be gained. Romanov *et al.* (1996) suggested that local chickens might contain genes and alleles pertinent to their adaptation to particular environmental conditions and local breeding goals. Therefore, maintaining local breeds is needed to permit genetic adaptation of populations to unforeseen breeding requirements in the future and as a source of research material (Horst, 1989; Besbes, 2009 and Tixier-Boichard *et al.*, 2009). The erosion of local chicken populations may be linked to the loss of valuable genetic variability and unique characteristics. The convention on biological diversity (<http://www.biodiv.org>) has put the need to conserve farm animal genetic diversity on the agenda. In farm animal diversity conservation, a unified approach accounting for two main roads to conservation has been established. This includes prevention of breed extinction and management of genetic diversity (Simianer, 2005).

There is a growing recognition that preservation of local chicken breeds is not only important to ensure the livelihoods of poor farmers who depend on these breeds, but their conservation is regarded as a national policy, as locally adapted chicken genetic resources could become future assets in breeding programs. Decision making in conservation requires specification of model parameters such as diversity, breed values, extinction probabilities and conservation potentials (Simianer, 2005). Beside phenotypic characterization, assessment of genetic characterization of local breeds is a prerequisite for this purpose (Wollny, 2003). Efforts should be made to preserve the important and unique characteristics that Vietnamese local chicken genetic resources possess. Genotypic characterization and conservation priorities in Vietnamese local chicken populations therefore need urgent attention.

Role of chickens in smallholder farming

In developing countries nearly all families at the village level, even the poor and landless, own poultry (Mack *et al.*, 2005). Major initiatives have been undertaken to develop

poultry as a tool for rural development. Poultry constitutes an important contribution to rural household's food security and income generation (Kitalyi and Mayer, 1998; Coplan and Alders, 2005; Alders and Pym, 2010). They are used to address gender inequalities. Women have more control and decision making powers on chickens than men (Kitalyi and Mayer, 1998; Guèye, 2000). In addition, village chickens are required for special festivals and essential for many traditional ceremonies (Coplan and Alder, 2005; Alders and Pym, 2010). Furthermore, chickens show the greatest variability of population types and make an important contribution to biodiversity (Tixier-Boichard *et al.*, 2009).

In Vietnam, chicken accounts for 70% of total poultry population. In 2006, the total poultry meat production (slaughtered poultry) was estimated to be 344.4 thousand tons and the number of eggs produced was 3.97 billions (GSO, 2007). Tung and Rasmussen (2005) showed that 31.7% and 17.8% of the total output of poultry production was consumed by semi-subsistence and semi-commercial poultry keepers, respectively. Poultry used as a source of protein to improve the nutrition for Vietnamese was reported by Epprecht (2005). The most important livestock-based source of income for the poorest income quintile is derived from poultry. Epprecht (2005) and Epprecht *et al.* (2007) reported that poultry accounts for about one quarter of the total household's income from livestock and further indicated that poultry serves as a 'sell-for-cash' tool for poor households.

Cuc *et al.* (2006) suggested a shift in gender ratio in chicken production due to the ownership of Vietnamese H'mong chickens by women. A similar observation was found by Burgos *et al.* (2008) who recognized the importance of Vietnamese poultry for children and women. The Vietnamese local chicken breeds also are an essential part of cultural and social activities (Vang, 2003 and FAO, 2009), for example Ho chicken are used for entertainment in religious celebrations and Ac and H'mong chicken are used for traditional medical purposes.

Chicken genetic diversity and assessment of genetic diversity

In developing countries, it had been widely assumed that local chickens have adapted to their local production systems which often are characterised by a limited supply of

resources and a lack of proper management program. They may present a diverse gene pool that could comprise unique genetic features. Some information on the genetic make-up of local chickens was reported (Horst, 1989). Thereby, their major genes with important effects on tropical oriented breeding already proved for their special utility in the tropics, such as dwarf (Dw), naked neck (Na), frizzle (E), silky (H), non-inhibitor (Id), fibro-melanosis (Fm), pea comb (P), blue shell (O) and slow feathering (K).

A wide diversity of indigenous chicken breeds in the tropics could form the basis for genetic improvement and diversification to produce more productive breeds adapted to specific environments and requirements (Horst, 1989). Therefore, the estimation of genetic diversity of the local chickens should be carried out to support conservation strategies and utilisations of their performance values.

Microsatellites and mitochondrial DNA (mtDNA) sequences have already proved to be useful for assessing genetic variability, while single nucleotide polymorphisms (SNPs) are becoming more and more popular due to their very high density and availability of high throughput genotyping techniques. Microsatellites are tandem repeats in the genomic DNA with very short (1-5bp) simple sequence motifs, and hence they are autosomally inherited (Tautz, 1989). Major advantages of these highly polymorphic markers are their locus specificity, abundance and random distribution over the genome, co-dominant inheritance, ease and speed of their application and suitability for semi-automated analysis (Weigend and Romanov, 2001). Unlike microsatellite markers, mtDNA is maternally inherited. The mtDNA is a circular molecule of 16,785 bp in size (Desjardins and Morais, 1990). The displacement loop (D-loop) region of the mtDNA contains elements that control the replication of the molecule and is highly polymorphic. MtDNA is used to infer regions of domestication and to identify the number of maternal lineages and their geographic origins (FAO, 2007b).

A combination of these two markers is a complementary approach that combines the highly polymorphic microsatellites whose high mutation rates allow for small-scale resolution of more recent demographic events with mtDNA which shed light on phylogeographic events dating further back in time (Feulner *et al.*, 2004). An assessment of genetic structure based on these two markers with different modes of inheritance provides more insights into the evolutionary forces shaping genetic diversity.

Total genetic diversity includes within and between breed diversity. Genetic diversity within a breed can be estimated by the number of alleles, the expected heterozygosity (Frankham *et al.*, 2002) and marker estimated kinships within a breed (Eding and Meuwissen, 2001). Genetic diversity between breeds can be assessed by various measures. A parameter for assessing diversity between breeds is the genetic differentiation or fixation indices which reveal the partitioning of genetic diversity (Wright, 1969). A wide range of studies for the assessment of genetic diversity were conducted using genetic distances (Nei, 1972 and Reynolds *et al.*, 1983). A unified approach to assess genetic diversity within and between populations is based on marker estimated kinships (Eding and Meuwissen, 2001). Bayesian clustering approaches have been suggested for admixture analysis of different populations (Pritchard *et al.*, 2000). This approach has already been proven to be useful to study the structure of populations of various farm animals (Rosenberg *et al.*, 2001; Fabuel *et al.*, 2004; Granevitz *et al.*, 2009; Leroy *et al.*, 2009; Li and Kantanen, 2009).

Recently, assessments of genetic diversity of chicken populations using the same set of microsatellite markers suggested by FAO (2004) have been published in several studies (Cuc *et al.*, 2006; Hillel *et al.*, 2007; Muchadeyi *et al.*, 2007; Chen *et al.*, 2008; Granevitze *et al.*, 2007; 2009 and Bodzsar *et al.*, 2009). Using the same markers in these studies allows unbiased comparisons. In a large scale study including 64 chicken populations from various continents and management systems, Granevitze *et al.* (2007) found considerable variation of within breed diversity (i.e., numbers of alleles/population and heterozygosity values). This variation reflected differences in population history and management. The average number of alleles and expected heterozygosity (3.6 ± 0.87 alleles and 0.51 ± 0.07 , respectively) was rather lower than that observed in human (Ayub *et al.*, 2003), cattle (Sodhi *et al.*, 2005) and pig (Behl *et al.*, 2006). Highest levels of within-population diversity were found in non-managed local populations, in some standardised breeds kept with a large population size and in some commercial broiler lines. A wide variation of within breed diversity was found for European fancy breeds. White-egg layer lines revealed the lowest level of diversity of all commercial lines. Therefore, white-egg layer lines might be considered to be in a more critical situation than other commercial lines concerning their future development, while some local breeds do represent an important reservoir of genetic diversity.

In the analysis of the population structure of a wide range of chicken breeds and lines from various continents and management systems, Granevitze *et al.* (2009) found six main clusters which were related to their geographical origins and histories. These six main clusters were formed by 1) brown egg layers, 2) predominantly broilers, 3) native Chinese breeds or breeds with recent Asian origin, 4) predominantly breeds of European derivation, 5) populations with no known history, and 6) the other populations shared their genome with some clusters defined as “Multi-clusters”. Within a country, different observations on population structure were revealed. Chen *et al.* (2008) and Bodzsar *et al.* (2009) found sub-structuring in Chinese and Hungarian chicken breeds, respectively, while Zimbabwean chicken populations do not exhibit a typical breed structure (Muchadeyi *et al.*, 2007). This implies a higher level of gene flow among agro-ecological zones or populations in the African country (i.e., Zimbabwe) than Asian and European countries (i.e., Vietnam and Hungary, respectively).

Some studies on Vietnamese chicken populations employing microsatellite data were published (Cuc *et al.*, 2006 and Berthouly *et al.*, 2009). These studies were carried out in a single province (i.e., Mai Son district, Son La province and Ha Giang province, respectively). The populations in these studies showed high diversity and no substructure. In addition, the highest genetic diversity was found in a H’mong population when comparing it to a wide range of globally collected chicken breeds (Granevitze *et al.*, 2007).

In the analysis of mtDNA sequences, haplotype network analysis clusters individuals based on haplotypes they possess and indicates how different these haplotypes are from those in other individual. The median networks of haplotypes were generated by partitioning the groups of haplotypes to portray mtDNA relationships and infer about population expansion and domestication events (Bandelt *et al.*, 1995). The ancient haplotypes can be distinguished from young ones due to their higher frequencies and central positions surrounded by derived haplotypes in a star like topology (MacHugh and Bradley, 2001). Bandelt *et al.* (1995) showed that the median networks of haplotypes provide a much more useful and informative mitochondrial portrait of the populations concerned than can be obtained from other traditional tree building approaches such as maximum parsimony, maximum likelihood and distance methods.

Applying haplotype network analysis, Liu *et al.* (2006) and Oka *et al.* (2007) conclude multiple and independent domestication events in South China, Southeast Asia and the Indian subcontinent. Liu *et al.* (2006) revealed the nine divergent clades (named clade A–I) related to geographical distribution in the wide range of domestic chickens in Eurasian regions, whereas Oka *et al.* (2007) identified seven clades (named clade A–G) observed in Japanese chickens. Recent studies on Zimbabwean (Muchadeyi *et al.*, 2008) and Sri Lankan (Silva *et al.*, 2008) chickens using haplotype network analysis also support the hypothesis of multiple origins of domesticated chicken. The populations in these studies indicated a high level of genetic diversity. In the study of Liu *et al.* (2006), the Vietnamese chicken samples were assigned into two different clades G and I. Clade G have originated from Yunnan and/or surrounding areas of China while clade I have origin in Vietnam. However, the study of Liu *et al.* (2006) was based on only a small number (n = 3) of Vietnamese chicken samples.

Conservation of local chicken genetic resources

FAO (2007a) indicated that on a global level about 20 % of all recorded farm animal breeds are classified as being “at risk”. Chicken genetic resources are considered to be the most endangered with the highest proportion of breeds at risk (33% of all recorded chicken breeds), four percent of the breeds were reported as extinct, the status of 40% was unknown, and the remaining 23% of the breeds were not at risk. The main factors that result in a loss of local animal genetic resources are low performance of local breeds, farmers' preferences for exotic breeds, the lack of adequate conservation policies, socio-economic changes, natural and human induced disasters, and the transformation of traditional systems into external input-oriented systems (FAO, 2007a).

Conservation and development of local breeds is important because of their contribution to the livelihoods of farmers and biodiversity as well as their social and cultural importance (FAO, 2007b). The need to conserve farm animal biodiversity is accepted by many countries through the ratification of the convention on biological diversity (<http://www.biodiv.org>). Recently, the global plan of action for animal genetic resources was adopted by 109 countries. These highly contributed to establish an effective

international framework for the sustainable use, development and conservation of animal genetic resources for food and agriculture, and world food security (FAO, 2007b). This is a major global thrust on genetic preservation and biodiversity which is reflected in efforts to further develop a global databank for animal genetic resources for food and agriculture genome and data banks (<http://dad.fao.org/>).

In Vietnam, the convention on biological diversity was adopted in 1995 (Tieu *et al.*, 2008). A total of 96 Vietnamese local livestock breeds including 11 chicken breeds are recorded in the FAO's global databank (<http://dad.fao.org/>). Information of these 11 breeds is given in Table 1. Within the country, the Vietnamese Government has issued a number of policies and decisions to support the management and to promote the development of animal production (Vang, 2003). Furthermore, national programs on conservation of the Vietnamese domestic animal genetic resources have been initiated under the direction of the National Institute of Animal Sciences (NIAS) since 1990. These policies and national programs have had positive impacts on the use and conservation of farm animal genetic resources. For example, several breeds (i.e, H'mong and Te chicken) were detected; two breeds (Ho and Te chicken) were rescued from risk of extinction; utilisation of Ac and H'mong chickens as a traditional medicine has been developed for human demand across the country (Tieu *et al.*, 2008). Almost all of the conservation programs implemented by NIAS have been conducted on farms with farmer participation (Tieu *et al.*, 2008). Farmers have been paid only 20% of total expenditure to maintain the breeds. Such a low allocation might not ensure to prevent the loss of national animal genetic resources in the long term (Tieu *et al.*, 2008).

Table 1. Information of 11 Vietnamese local chicken breeds.

Breed	Origin	Distribution	Adult body weight (kg)		Egg yield/ year (eggs)	Special feature	Status
			Male	Female			
H'mong	Northwest and North Central Coast	North and Centre	2.2 - 2.5	1.6 - 2.0	60 - 75	Traditional medicine	Decreased
Oke	Northeast	Northeast	2.5	1.2	24 - 30	Traditional medicine	Decreased
Ho	Red River Delta	Red River Delta	4.5 - 5.5	3.5 - 4.0	45 - 60	Beautiful appearance	Decreased
Dong Tao	Red River Delta	Red River Delta	4.5	3.5	70	Short and thick legs	Increased
Ri	Red River Delta	North and Centre	2.7	1.2	60 - 75		Decreased
Mia	Red River Delta	Red River Delta	3.0	2.3	55 - 60		Increased
Te	North and Centre	North and Centre	1.6	1.3	54 - 72	Dwarfism	Endangered
Tre	South Central Coast	Across the country	1.2 - 1.3	0.8 - 0.9	56 - 70	Game cock	Decreased
Choi	South Central Coast	Across the country	4 - 5	3.5 - 4	32 - 40	Game cock	Decreased
Tau Vang	Mekong Delta	South	2.0	1.4	90 - 120		Increased
Ac	Mekong Delta	North and South	0.70 - 0.75	0.55 - 0.60	90 - 100	Traditional medicine	Increased

(<http://dad.fao.org/>; NIAS, 2000 and Su *et al.*, 2004)

Conservation strategy involves the identification and prioritization of targets for conservation. One of the objectives of conservation programs is to maintain genetic diversity for potential future use. Eding and Bennewitz (2007) indicated two different methods using molecular genetic variation to derive conservation priorities which are the core set (Eding *et al.*, 2002) and the Weitzman (1992)'s methods.

The basis principle of the core set method is the elimination of genetic overlap in the total set of populations. The genetic overlap or genetic similarity between individuals and populations is described by the coefficient of kinship (Eding and Bennewitz, 2007). If the average kinships between and within breeds are known, the average kinship in a core set is calculated and the contribution of each breed to the core set is estimated. In the case of the core set, these contributions are chosen such that the average kinship in the core set is minimized. By estimating contributions to a core set, the populations under study are ranked according to their genetic uniqueness. From this, the relative importance of populations in conserving the genetic diversity is found (Eding *et al.*, 2002).

A core set can be thought of as a live or cryo-conserved mixed population, which is composed of various proportions of different breeds. The breed contributions to the core set are derived in such a way, that the expected diversity of the total core set is maximized. When the objective of the conservation measures is to maximize conserved genetic variation, then the core set method should be used (Bennewitz *et al.*, 2007). While this approach combines both between and within-breed diversities, non-genetic criteria like any sort of specific breed value or degree of endangerment are not taken into account. Therefore, using this method could ignore risk of extinction of the breed which plays an important role in conservation decision (Simianer, 2005 and FAO, 2007a). In addition, the method likely increases the opportunity for genetic flow, and chances to lose unique alleles from the population (Tapio *et al.*, 2006).

A widely used formal approach for decision making in livestock conservation is based on Weitzman's (1992) diversity concept. The Weitzman diversity is a diversity of elements which are homogeneous and pair-wise clearly distinct. The approach aims to maximize expected diversity and uses the conservation potential, which combines the genetic diversity between breeds and their extinction probabilities estimated by socio-economic

factors to prioritize breeds for conservation (Simianer, 2005). Conservation potential is considered as the single most useful breed alert indicator in conservation decision (Weitzman, 1993). Conservation potentials were applied for prioritizing cattle (Simianer *et al.*, 2003 and Zerabruk *et al.*, 2007) and chicken (Pinent *et al.*, 2005) breeds. The populations in these studies showed a considerable variability of their conservation potentials.

Although migration between breeds or within breed diversity is neglected applying the Weitzman approach as criticized by Caballero and Toro (2002) and Eding *et al.* (2002), decisions on investments in conservation based on the Weitzman approach are driven not only by the phylogenetic substructure, but also by economic and social factors, which serve as an important consideration in the conservation decisions (Reist-Marti *et al.*, 2003; Simianer *et al.*, 2003; Pinent *et al.*, 2005 and FAO, 2007a). In addition, applying the Weitzman diversity in conservation strategy can maintain allelic diversity within species, ensuring access maximum adaptive potential of species (Simianer, 2005 and Tapio *et al.*, 2006) since conservation activities aim to reduce the extinction probability and to increase expected diversity of populations as suggested by Simianer (2005) and FAO (2007a).

Conservation strategies also require consideration on how the available resources should be distributed among the breeds under consideration, and decisions as to which is the most efficient conservation strategy among several options available (FAO, 2007a). As a total budget for these activities is limited, the key questions are how many and which breeds will receive financial support and how to allocate the funding in the most optimal way. In Vietnam, national conservation programs financially support farmers to maintain their breeds despite the economic inferiority of their breeds. Such a low allocation might not ensure to prevent the loss of national animal genetic resources in the long term (Tieu *et al.*, 2008).

Simianer (2002) showed that an optimum allocation can substantially increase the efficiency of the use of available conservation funds. Furthermore, Simianer *et al.* (2003) proposed an algorithm to calculate optimum allocation of resources to a set of breeds based on three models (named model A, B and C) with different assumptions. Model A assumes that a fixed amount of money per year pays a premium to owner of chickens,

balancing some of the economic inferiority of those chickens, which reflects the present scene established in Vietnam. Model B presumes that a basic strategy for maintaining diversity in small population is to keep family size constant. When chickens are raised for replacement, this information is brought to the chicken owners by extension specialists. With more animals more extension specialists are needed. In this case, the costs are proportional to the population size. Model C is based on the assumption that the information on within breed conservation measures can be brought to the chicken owners through some established information channels without any expenditure. Therefore, the costs are independent on the population size, but are a function of the number of populations. These assumptions cover a wide range of possible conservation policies, and so the application of all three models will identify the relevant range of sensible allocation patterns. In an application to African cattle data, Simianer *et al.* (2003) showed that using the optimum allocation approaches can double the cost efficiency of conservation programs, but the choice of the different models did not strongly affect the outcome.

The agro-ecological zones and local chicken breeds in Vietnam

Vietnam is a tropical country located in the centre of Southeast Asia. The country stretches 1 600 km from North to South, between 8°33' and 23° 20' North and 102° and 109° 27' East. The total area is 329 566 km². The climate varies greatly from North to South. The country is divided into eight agro-ecological zones, based on topography, soil pattern, and climate (Figure 1). The rainfall, temperature, major topographic features and farming systems of each agro-ecological zone are given in Table 2 (Vang, 2003; FAO, 2005 and GSO, 2007). The diversity of natural environment and differences in production systems in the country has contributed to the differentiation of local chicken breeds (BMBF, 2006). FAO (<http://dad.fao.org>) reported 11 Vietnamese indigenous chicken breeds besides one extinct (Van Phu breed). From these 11 breeds, one breed (Te chickens) is critically endangered. Six breeds have been decreasing in numbers while the other remaining breeds have been increasing in numbers (NIAS, 2000). These 11 breeds have originated and are distributed in the different agro-ecological zones. Five breeds have originated from Northern Vietnam, two breeds have origins in the centre of

Vietnam, two breeds have their roots of both the north and centre of Vietnam and two breeds have originated from the South (Table 1).

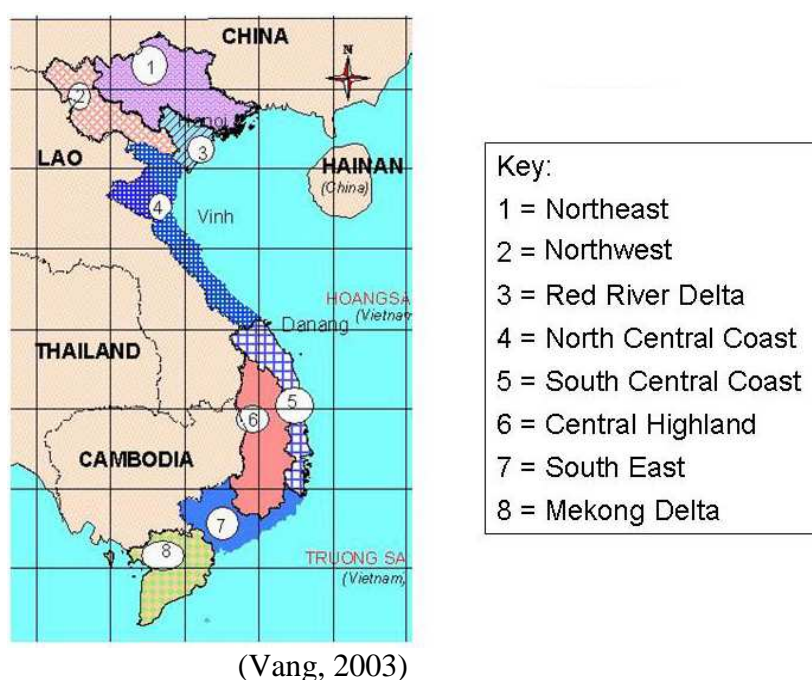


Figure 1. Map of Vietnam and eight agro-ecological zones (free scale).

Table 2. The rainfall, temperature, major topographic features and farming systems of each agro-ecological zone.

Zone	Area (km ²)	Rainfall (mm/year)	Temperature (°C)	Physical regions	Farming system
Northeast	6 402	1 721	23.2	Mountain and midland	Extensive
Northwest	3 754	2 355	21.8	Mountain and midland	Extensive
Red River Delta	1 486	2 013	23.3	Lowland	Semi-extensive
North Central Coast	5 155	2 985	24.1	Mountain, lowland and sandland	Extensive
South Central Coast	3 316	2 389	26.3	Mountain, lowland and sandland	Extensive
Central highland	5 466	1 611	19.9	Highland	Semi-extensive
South East	3 481	1 390	27.8	Lowland	Intensive
Mekong delta	4 061	2 679	27.1	Lowland	Semi-extensive

(Vang, 2003; FAO, 2005 and GSO, 2007)

Although 11 Vietnamese local chicken breeds have been reported, the definition of these breeds is not fully standardized. It appears rather unlikely that valid results based on phenotypic characterization programs may be obtained for local chicken breeds considering the various local production systems. Furthermore, these chickens are reared in isolated villages. There is a need to investigate genetic diversity between and within local breeds to develop effective conservation programs and to determine whether the chicken populations in different areas represent unique populations. Conservation potential for each breed needs to be calculated for the definition of conservation priorities. This study is also necessary to maximize efficiency of funding allocation for conservation.

Scope of the thesis

The major scope of this thesis is to characterize genetic diversity of Vietnamese local chicken breeds and to identify population priorities for conservation. In particular, this thesis aims at

- i. Evaluating genetic diversity within and between Vietnamese local chicken breeds of diverse geographical origins based on different molecular markers.
- ii. Characterising the Vietnamese chicken gene pool relative to the Chinese chicken breeds and estimating the degree of uniqueness of the Vietnamese breeds to total diversity of a set of three Red Jungle fowl populations.
- iii. Estimating the conservation potential of Vietnamese local chicken breeds.
- iv. Investigating optimal allocation of conservation funds to minimize loss of genetic diversity between these breeds.

The first issue is covered in chapters 2 and 3. The second issue is presented in chapter 4. Chapter 5 contains the third and fourth issues, while the general discussion is presented in the final chapter 6.

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2nd CHAPTER**Assessing genetic diversity of Vietnamese local chicken breeds using
microsatellites**

N. T. K. Cuc^{1,2,3}, H. Simianer¹, H. Eding², H. V. Tieu³, V. C. Cuong³, C. B. A. Wollny⁴,
L. F. Groeneveld² and S. Weigend²

¹Department of Animal Sciences, Animal Breeding and Genetics Group, Georg-August-Universität Göttingen, Göttingen, Germany, ²Institute of Farm Animal Genetics, Friedrich- Loeffler-Institut, Neustadt-Mariensee, Germany, ³National Institute of Animal Sciences , Hanoi, Vietnam. ⁴Faculty of Life Sciences, University of Applied Sciences Bingen, Bingen, Germany

Animal Genetics (in press)

Summary

This study aims to assess genetic diversity within and between nine Vietnamese local chicken breeds and two Chinese breeds included for comparison. Genotyping 29 microsatellites revealed high diversity of both Vietnamese and Chinese breeds. Cluster analysis using the STRUCTURE software suggested six clusters as most likely grouping of the 11 breeds studied. These groups encompassed four homogeneous clusters, one formed by the two Chinese breeds and the other three representing a single breed each: the Mekong Delta breed Ac, the South Central Coast breed Choi, and the Red River Delta breed Dong Tao. The six remaining breeds formed two additional admixed clusters.

Keywords: genetic diversity, phylogenetic structure, Vietnamese local chickens, microsatellites.

Introduction

In Vietnam chicken population was estimated 152.7 millions in 2006. The distribution varies considerably. In the northern part, it ranges from 2.9 million in the Northwest to 40.6 million birds in the Red River Delta while in the southern part, a total of 9.6 million and 19.8 million chickens was found in the South Central Coast and the Mekong Delta, respectively (Desvaux *et al.*, 2008). According to Hong Hanh *et al.* (2007), more than 70% of the country's total chicken population are local chickens. Vietnamese local chicken breeds are defined mainly based on phenotypic characteristics. Little is known about the genetic relationships between them. The quantification of genetic diversity is a prerequisite to develop effective conservation programs, and molecular markers have been shown to provide useful information (Soller *et al.*, 2006). Recent studies of Vietnamese chicken populations employing microsatellite data showed high diversity and no population substructure (Cuc *et al.*, 2006 and Berthouly *et al.*, 2009). However, these studies were limited to a single district (Mai Son of the Son La Province, Cuc *et al.*, 2006) or region (Ha Giang province, Berthouly *et al.*, 2009).

To achieve a more comprehensive picture of the genetic diversity of Vietnamese chickens, the current study was carried out on a wider range of local Vietnamese breeds

kept across the country. This study assesses the degree of diversity within and relationships between nine of 11 Vietnamese local chicken breeds recorded by FAO (DAD-IS, <http://dad.fao.org>). The H'mong breed was included in the study of Cuc *et al.* (2006) while eight additional Vietnamese local chicken breeds were added. These nine breeds are located in eight different districts in four agro-ecological zones from both the southern and northern parts of Vietnam (Table 1) which were reported as areas of origin or current distribution of the populations under study (Su *et al.*, 2004). On average, blood from 32 randomly selected individuals per breed was collected by sampling one male and one female per household. Except for Te breed which only eight samples collected from four households due to their rareness and the other 24 samples were sampled from conservation flock at the National Institute of Animal Sciences (NIAS). Two Chinese breeds (16 males and 16 females per breed), the Tam Hoang and Luong Phuong, were included for comparison. These breeds were imported into Vietnam in 1995 and 2003, respectively, and have been kept as conservation flocks at the NIAS. They are most common exotic breeds kept as scavenging chickens in Vietnam and it is assumed that Chinese chickens from NIAS have been introgressed to Vietnamese local chicken gene pool (Tieu *et al.*, 2008). DNA polymorphism was assessed by genotyping a total of 353 individuals at 29 microsatellite loci as described by Granevitze *et al.* (2007).

Genetic diversity was assessed by calculating the number of alleles per breed, and expected and observed heterozygosities with the FSTAT software (Goudet, 2001). Population structure was determined using the Bayesian model-based clustering as implemented in the STRUCTURE software (Pritchard *et al.*, 2000). Individuals were grouped into a predefined number of K clusters ($2 \leq K \leq 8$) with 100 independent runs for each K value. The SIMCOEFF software (Rosenberg *et al.*, 2002) was used to compare repeated runs. Solutions with a similarity coefficient higher than 95% were considered as identical. The most frequent solution was visualised using DISTRUCT software (Rosenberg, 2004). In addition, the approach developed by Evanno *et al.* (2005) was applied from K = 2 to K = 9 to determine the optimal number of clusters.

The average number of 6.09 ± 0.67 alleles per breed and the average expected heterozygosity of 0.634 ± 0.034 in the current study are in agreement with previous findings which indicated high diversity of Vietnamese chickens (Table 1). Analysing a wide range of chicken populations originating from various continents and management

systems, Granevitze *et al.* (2007) found mean allele numbers and expected heterozygosity estimates per population varying from 2.30 to 6.72 and 0.28 to 0.67, respectively, with the Vietnamese H'mong breed being the most variable one. Berthouly *et al.* (2009) reported that the mean expected heterozygosity of Vietnamese chickens in the Ha Giang province was 0.62, while the corresponding values for Red Jungle Fowl, Chinese and commercial breeds were 0.60, 0.47 and 0.40, respectively.

The results of the STRUCTURE analysis from $K = 2$ to $K = 6$ are shown in Figure 1. Except for $K = 2$, the highest repeatability of identical runs, as identified by SIMCOEFF, was at $K = 6$, and declined strongly at higher K values (data not shown). Using the method developed by Evanno *et al.* (2005) also identified six clusters as most probable solution. These clusters encompassed four homogenous; one formed by the two Chinese breeds and another three representing a single breed each: the Mekong Delta breed Ac, the South Central Coast breed Choi and the Red River Delta breed Dong Tao. The other two admixed clusters included the six remaining breeds, in which the Mekong Delta breed Tau Vang showed an admixture between two Chinese and Vietnamese local breeds. This finding is consistent with the Chinese origin of Tau Vang chickens (Linh, 2005). For all K values, the two Chinese breeds clustered separately from the Vietnamese local chicken breeds indicating that the Vietnamese local chicken breeds make up a gene pool which is different from the two Chinese ones. Within Vietnam, the chicken breeds from the northern part make up one unstructured gene pool. This is in agreement with previous studies (Cuc *et al.*, 2006 and Berthouly *et al.*, 2009). In contrast, differentiation of the Vietnamese chicken breeds are observed between the northern part and South Central Coast as well as Mekong Delta, indicating sub-structuring of the Vietnamese chicken breeds related to their distant geographical distribution.

Table 1. The average number of alleles/locus, expected (H_E) and observed (H_O) heterozygosity of nine Vietnamese and two Chinese breeds.

Breed	Agro-ecological zone Study area		No. of blood samples	Alleles/locus	$H_E \pm SD$	$H_O \pm SD$	
Vietnamese	H'mong	Northwest	Mai Son, Son La	31	6.48 ± 3.07	0.657 ± 0.028	0.633 ± 0.016
	Mia	Red River Delta	Duong Lam, Ha Tay	32	6.69 ± 3.43	0.646 ± 0.033	0.610 ± 0.016
	Ri		Hoai Duc, Ha Tay	32	7.14 ± 3.81	0.648 ± 0.031	0.606 ± 0.016
	Ho		Thuan Thanh, Bac Ninh	32	5.76 ± 2.64	0.618 ± 0.034	0.564 ± 0.016
	Dong Tao		Khoai Chau, Hung Yen	32	5.31 ± 0.36	0.573 ± 0.035	0.548 ± 0.016
	Te		Ba Vi, Ha Tay	32	5.55 ± 2.32	0.635 ± 0.029	0.595 ± 0.016
			NIAS				
	Choi	South Central Coast	Ninh Hoa, Khanh Hoa	33	5.66 ± 2.98	0.623 ± 0.035	0.645 ± 0.016
	Ac	Mekong Delta	Tan an, Long An	32	5.48 ± 2.50	0.610 ± 0.033	0.608 ± 0.016
	Tau Vang			33	6.72 ± 3.27	0.696 ± 0.021	0.563 ± 0.016
	Mean		32	6.09 ± 0.67	0.634 ± 0.034	0.600 ± 0.033	
Chinese	Luong Phuong	(imported from	NIAS	32	5.21 ± 2.23	0.680 ± 0.023	0.657 ± 0.017
	Tam Hoang	China)		32	4.48 ± 1.77	0.627 ± 0.023	0.606 ± 0.016
	Mean			32	4.85 ± 0.52	0.654 ± 0.037	0.632 ± 0.036

Key: NIAS = National Institute of Animal Sciences

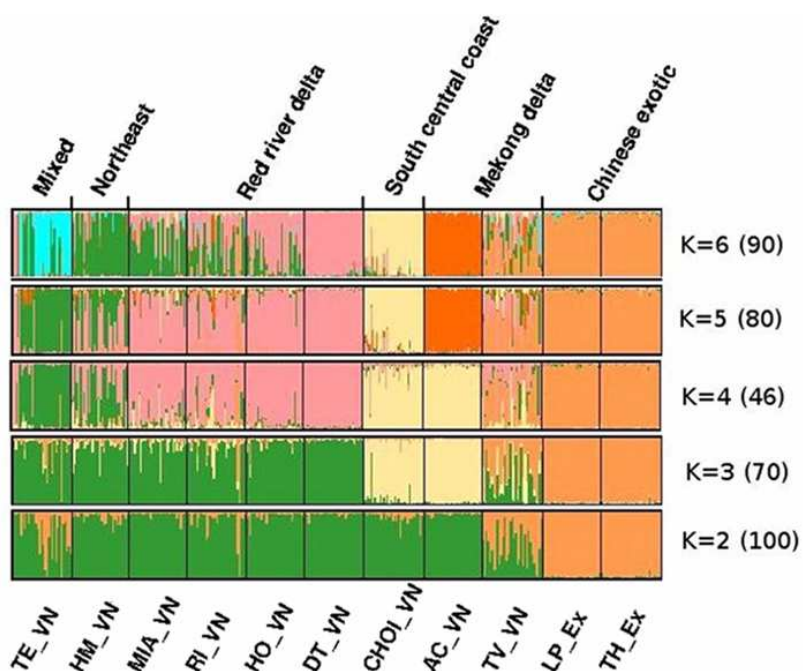


Figure 1. Substructure of nine Vietnamese local and two Chinese chicken populations. Number in parenthesis is the number of identical solutions of 100 repeats within each number of K clusters at 95% threshold.

Key: TE_VN = Te; HM_VN = H'mong; MIA_VN = Mia; RI_VN = Ri; HO_VN = Ho; DT_VN = Dong Tao; CHOI_VN = Choi; AC_VN = Ac; TV_VN = Tau Vang; LP_Ex = Luong Phuong; TH_Ex = Tam Hoang.

Acknowledgements

The authors express sincere thanks to the Vietnamese farmers for providing samples and to A. Weigend and A. Flörke, Institute of Farm Animal Genetics at Mariensee, for technical assistance. N.T.K. Cuc had a stipend from German Academic Exchange Service, and the project was funded by the German Federal Ministry of Education and Research.

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3rd CHAPTER

Multiple maternal lineages of Vietnamese local chickens inferred by mitochondrial DNA D-loop sequences

N. T. K. Cuc^{1,2}, H. Simianer¹, L. F. Groeneveld² and S. Weigend²

¹Department of Animal Sciences, Animal Breeding and Genetics Group, Georg-August-Universität Göttingen, Göttingen, Germany, ²Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt-Mariensee, Germany.

Submitted for publication

Summary

In this study, mitochondrial DNA (mtDNA) sequence polymorphism was used to assess genetic diversity of nine Vietnamese local chicken breeds. In addition, two Chinese breeds kept in Vietnam were included in the analysis for comparison. A 455-bp fragment of the mtDNA D-loop region was sequenced in 222 chickens of these 11 breeds. As reference, a skeleton was constructed based on chicken mtDNA sequences taken from the Genbank. Haplotypes of the nine Vietnamese local and two Chinese breeds were aligned together with these sequences. The Vietnamese and Chinese breeds showed a high degree of variability. In total, 37 haplotypes were identified in the chicken breeds studied forming eight clades. Thereby, the majority of individuals of the two Chinese breeds grouped together in one clade which is assumed to have its roots in the Indian subcontinent. Although the Vietnamese chicken breeds were distributed across all eight clades, most of them clustered in three main clades. These results suggest that the Vietnamese domestic chickens have originated from multiple maternal lineages, presumably originating from Yunnan and adjacent areas in China, South and Southwest China and/or surrounding regions (i.e., Vietnam, Burma, Thailand, and India).

Keywords: mitochondrial DNA, Vietnamese local chickens, maternal lineages.

Introduction

Mitochondrial DNA is considered as a powerful source of molecular information to track the ancestry of breeds back hundreds of generations (Harpending *et al.*, 1998). Different hypotheses about chicken domestication based on mtDNA analyses are found in the literature. Fumihito *et al.* (1996) suggested a monophyletic origin of domestic chickens from *Gallus gallus gallus* and a single domestication event occurred in Thailand and adjacent regions while Kanginakudru *et al.* (2008) found evidence for domestication of Indian chickens from *Gallus gallus spadiceus* and *Gallus gallus gallus* as well as from *Gallus gallus murghi*. A study that analyzed BCDO2, a gene encoding an enzyme which converts carotene into its colourless variant, suggested the introgression of the yellow skin gene from *Gallus sonneratii* into modern chicken breeds (Eriksson *et al.*, 2008). Other reports assumed multiple and independent domestication events in South China,

Southeast Asia and the Indian subcontinent (Liu *et al.*, 2006 and Oka *et al.*, 2007). Liu *et al.* (2006) revealed nine divergent clades (named clades A–I) related to geographical distribution in a wide range of domestic chickens in Eurasian regions. Oka *et al.* (2007) identified seven clades (named clades A–G) observed in the Japanese chickens. Of the seven clades of Oka *et al.* (2007), the four clades A, B, C and E are identical to clades E, A, D and B described by Liu *et al.* (2006), respectively.

Vietnam is located in Southeast Asia having a common border of 1 350 km with Southern China (GSO, 2008). Human migration from Southern China to Vietnam enabled introduction of Chinese chickens to Vietnam (Taylor, 1983; Nyiri and Saveliev, 2002). In addition, during the recent years, some exotic breeds were imported. Farmers' preferences for exotic breeds with high performance may have led to introgression of exotic breeds into the Vietnamese gene pool (Tieu *et al.*, 2008). Little is known, however, about the origin and the history of Vietnamese domestic chickens. Some recent studies based on microsatellite markers showed sub-structuring of Vietnamese chickens (Cuc *et al.*, 2010) and a close relationship between the Vietnamese chickens of the Ha Giang province with Red Jungle fowl populations (Berthouly *et al.*, 2009). Based on mtDNA analysis, Liu *et al.* (2006) found the distribution of the Vietnamese chicken samples in two different clades. The latter study, however, was based only on a small number ($n = 3$) of Vietnamese chicken samples. The current study aims to determine the degree of shared maternal mtDNA haplotypes between Vietnamese local chicken breeds, and hence to reveal maternal lineages of origin.

Materials and methods

Populations and blood sampling

The study was carried out in nine Vietnamese local chicken breeds. These breeds are located in eight different districts in four agro-ecological zones from both the southern and northern parts of Vietnam. The Ho breed was kept in only one village (Ho Village) in the Red River Delta in the North whereas the Ri and Tau Vang breeds are raised in almost every household in the North and in the South, respectively. Two breeds, Dong Tao and Mia, are kept in the Red River Delta. The Te chickens are assumed to carry a dwarf gene.

However, information on the distribution of these chickens in Vietnam is not available. The Choi chickens are game birds reared at many places across the country. The Ac and H'mong chickens, mainly kept in the South and North, respectively, are used for making traditional tonics to treat old or sick people. The Tam Hoang and Luong Phuong Chinese breeds, which were included for comparison were imported into Vietnam in 1995 and 2003, respectively, and have been kept as conservation flocks at the National Institute of Animal Sciences (NIAS) since that time. On average, blood samples were taken from 20 randomly selected individuals per Vietnamese breed. Thereby, one male and one female were sampled per household. In addition, blood samples from 21 birds of each of the Chinese breeds were collected.

MtDNA amplification and sequencing

A fragment of 455 bp from the mtDNA D-loop region was amplified using primers mtGlu-F (5'-GGCTTGAAAAGCCATTGTTG-3') and mtGlu-R (5'-CCCAAAAAGAGAAGGAACC-3'). Due to their circular nature, these primers are positioned at bases 16739 – 16775 (forward primer) and 649 – 668 (reverse primer) of the complete mtDNA sequence of domestic chickens (X52392, Desjardins and Morais, 1990). PCR amplifications and sequencing were done as described by Muchadeyi *et al.* (2008). To align DNA sequences, AlignIR software was used (LICOR Inc. Nebraska, USA). The list of sequences used in this study and the corresponding GenBank accession numbers are provided in Table S1.

Statistical analysis

The position and number of polymorphic sites as well as corresponding haplotypes were calculated using MEGA v. 3.1 (Kumar *et al.*, 2004). The distribution of haplotypes in the samples was computed using TCS v. 1.2.1 (Clement *et al.*, 2000). Median joining networks of haplotypes were constructed following the algorithm of Bandelt *et al.* (1995) and using NETWORK v. 4.5.1.0 (<http://www.fluxus-engineering.com/sharenet.htm>). As reference, network analysis was used first to create a skeleton which was based on the most frequent haplotypes of the nine clades of Liu's network (Liu *et al.*, 2006) and the three additional clades (D, G and F) of Oka *et al.* (2007). This skeleton assigns clades to suggested regions of domestication in chickens, which were Yunnan and/or surrounding

areas (Liu's clades A, B, F and G), South and Southwest China and/or surrounding areas and Southeast Asia (Liu's clade C, D, H, I and Oka's clade D, F, G), and the Indian subcontinent (Liu's clade E). Nomenclatures of the nine clades reported by Liu *et al.* (2006) were used as reference for the clade notation in this study. The sequences used for alignment consisted of 455 bp. Various networks were constructed by using different epsilon (ϵ) values ranging from zero to 20. There were no considerable differences among the different networks except a slight increase in the network connections where clades joined. The median network presented used an epsilon value of 5. The haplotype and nucleotide diversities of breeds were computed using ARLEQUIN v. 3.1 (Excoffier *et al.*, 2006).

To analyse if mtDNA clades also differed at the autosomal level, the data obtained from genotyping 29 microsatellite markers of these 222 individuals (Cuc *at al.*, 2010) was used. These individuals were labelled according to their clade affiliation based on mtDNA sequences. The microsatellite genotyping data were used in the Bayesian model-based clustering as implemented in STRUCTURE v. 2.3.1 to cluster individuals to a varying number of K clusters ($2 \leq K \leq 8$) (Pritchard *et al.*, 2000). Runs within each K-value showing a similarity coefficient of 0.95 and higher were considered as identical.

Results

Network profiles of the clades

The distribution of clades is shown in Figure 1. Eight clades A to G, and I were found in the chicken breeds studied. Clades A, B and E were the most frequent whereas clades F, G and I consisted of a small number of individuals only. Within each of three clades A, B and E, the major haplotypes were A1, B1 and E1 encompassing 70%, 77% and 57% of each clade, respectively. The major haplotype of clade D was D1, of which 50% was observed in the Choi breed (Table S2).

Breed distribution within clades

The Vietnamese local chickens were found in all eight clades (Figure 1). The distribution of the Vietnamese breeds into clades was not related to their geographical distribution.

The most frequent clades A and B included all nine Vietnamese breeds. A considerable proportion of Vietnamese local chickens belonged to clade D while only a small number of Vietnamese chicken was assigned to the five remaining clades (C, E, F, G and I). In contrast, the majority (76%) of Chinese chickens were found in clade E whereas no Chinese chickens were observed in clades D, G, F and I.

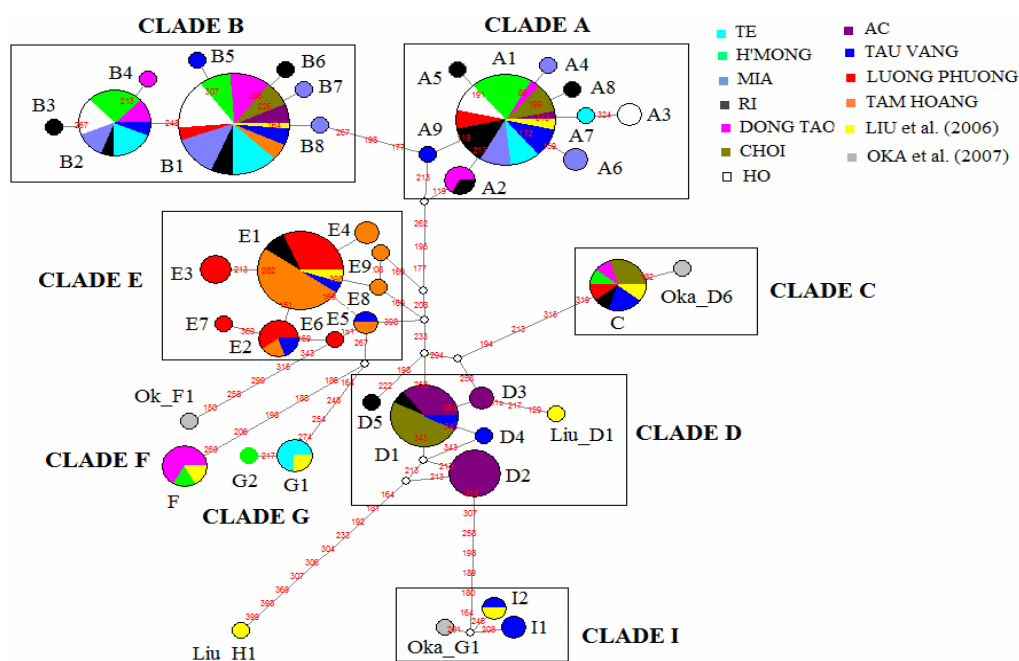


Figure 1. Median network profile of the mtDNA D-loop haplotypes observed in the current study. Data merged with sequences of major haplotypes reported by Liu *et al.* (2006) and Oka *et al.* (2007). The circle size corresponds to haplotype frequency, and the numbers on the line correspond to mutational positions connecting haplotypes. Empty circles are median vectors used in connecting indirectly related haplotypes.

Within-population diversity

Eight clades (A-G and I) were formed by 37 haplotypes, of which 25 were only found in the Vietnamese local breeds, six haplotypes were observed exclusively in the Chinese breeds, and the remaining six haplotypes were common (Table S3). The lowest haplotype diversity (0.615 ± 0.105) was estimated in the Ho breed, while the highest corresponding value (0.942 ± 0.034) was observed in the Tau Vang breed (Table 1).

Table 1. Polymorphic sites, haplotype and nucleotide diversity of chicken breeds under study.

Breed	Agro ecological zone	Study area	N	No. of Polymorphic sites	No. of Haplotypes	Haplotype diversity (\pm SD)
H'mong	Northwest	Mai Son, Son La	20	23	6	0.778 \pm 0.055
Mia	Red River Delta	Duong Lam, Ha Tay	20	10	7	0.737 \pm 0.094
Ri		Hoai Duc, Ha Tay	20	22	12	0.911 \pm 0.045
Ho		Thuan Thanh, Bac Ninh	20	8	4	0.615 \pm 0.105
Dong Tao		Khoai Chau, Hung Yen	20	20	7	0.768 \pm 0.080
Te		Ba Vi, Ha Tay and NIAS	20	14	5	0.716 \pm 0.086
Choi	South Central Coast	Ninh Hoa, Khanh Hoa	19	15	4	0.754 \pm 0.053
Ac	Mekong Delta	Tan an, Long An	21	13	5	0.767 \pm 0.053
Tau Vang			20	24	13	0.942 \pm 0.034
Luong Phuong	(imported from	NIAS	21	19	8	0.852 \pm 0.053
Tam Hoang	China)		21	11	7	0.705 \pm 0.095
Total			222	43	37	0.849 \pm 0.184

Relationship between mtDNA and autosomal genetic structure

The results of the STRUCTURE analysis from $K = 2$ to $K = 6$ are shown in Figure 2. The repeatability, i.e., the number of runs giving result with similarity coefficient ≥ 0.95 , varied from 34 to 100 from $K = 2$ to $K = 6$, while no identical runs were found at $K = 7$ and 8 (data not shown). For all K values, the mtDNA defined clade E was found as a pure cluster at the autosomal level while the other seven mtDNA defined clades were mixed to different degrees (Figure 2).

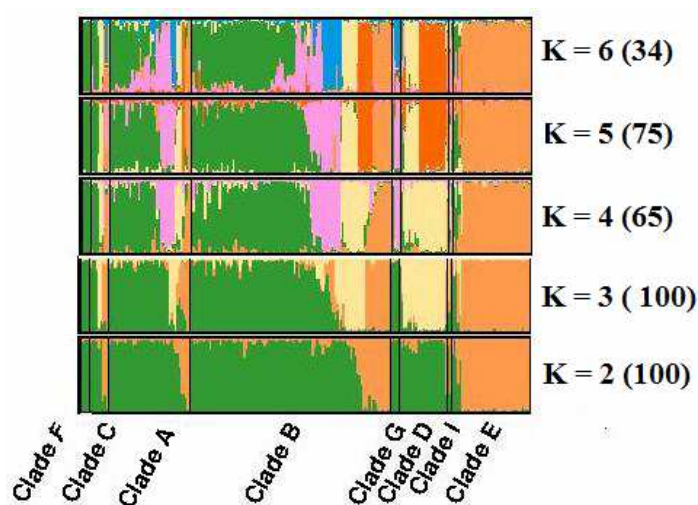


Figure 2. STRUCTURE based clustering using microsatellite genotypes of chicken groups assigned to eight mtDNA clades. Individuals are labelled according to their clade affiliation based on mtDNA sequences. Number in parenthesis is the number of runs giving an identical result (similarity coefficient ≥ 0.95).

Discussion

The majority of the Vietnamese local chickens carried mtDNA haplotypes that clustered in clades A and B. Based on the skeleton of supposed regions of domestication, this finding suggests the existence of two maternal lineages dominating in the Vietnamese local chickens which presumably originate from Yunnan and surrounding regions in China (Liu *et al.*, 2006). Fourteen percent of Vietnamese chickens were found in clade D indicating that this clade also contributed considerably to the Vietnamese local chickens.

Liu *et al.* (2006) and Oka *et al.* (2007) suggested that this clade has its root in Southeast Asia, South and Southwest China and/or surrounding areas (i.e., Vietnam, Burma, Thailand, and India). This finding would be in agreement with historical records of human immigration from southern China to Vietnam. Yüeh people are inhabitants in the Southeastern coast of China and are the ancestors of the Cantonese, i.e., Guangzhou and Guangxi Southern Chinese people. By the 3rd century B.C., Yüeh people emigrated from Southern China to the Red River Delta of Vietnam and mixed with the indigenous Van Lang Vietnamese population (Taylor, 1983). Additionally, Southern Chinese people from Yunnan, Guangzhou and Guangxi Provinces arrived at the North of Vietnam and moved to the South from the 17th to the 19th century A.C. (Nyiri and Saveliev, 2002). Descriptions of immigration always state that people of a family moved together with their animals which could result in the introduction of chickens from Southern China into the North and the South of Vietnam. While Yunnan, South and Southwest China might be seen as region of origin of the Vietnamese chicken breeds, the majority of individuals of Chinese breeds in this study were not assigned to these maternal lineages. This finding indicates that two Chinese breeds kept at NIAS do not represent the breeds of Yunnan, South and Southwest China.

The high proportion of haplotype D1 found in the Choi chickens is in agreement with findings of Liu *et al.* (2006), who reported that clade D mainly consisted of game birds. On the other hand, the clustering of the remaining Choi chickens in clades A and B is consistent with the study of Oka *et al.* (2007) who found game birds assigned to their clades B and E. Consequently, our findings suggest that the game breed Choi is a mixture of multiple maternal lineages.

A small number of Vietnamese chickens distributed in clades C, F, G and I indicates that these clades have little contribution to Vietnamese chickens. A small portion (2%) of Vietnamese local chickens was observed in clade E, originating from the Indian subcontinent (Liu *et al.*, 2006), which otherwise harboured mainly the Chinese chickens studied. Vietnamese local chickens in this clade included the Ri and Tau Vang breeds. This observation may indicate a possible exchange of genetic material between the Ri and Chinese chickens due to the wide distribution of the Ri chickens, while the Chinese origin of the Tau Vang breed (Linh, 2005) is known and explains the distribution of this breed in both Vietnamese and Chinese clades. This finding is also in agreement with the analysis

at the autosomal level in which the Tau Vang breed showed clear admixture between the Chinese and Vietnamese gene pools (Cuc *et al.*, 2010).

Although the majority of the Vietnamese chicken breeds in this study were assigned to clade A and B, the Vietnamese breeds were found to be highly polymorphic in the mtDNA D-loop region. Estimates of haplotype diversity ranged from 0.62 to 0.94 in this study and were higher than reported previously. Muchadeyi *et al.* (2008) found the haplotype diversity ranging from 0.61 to 0.73 and from 0.27 to 0.78 in Zimbabwean chickens and purebred lines, respectively. Liu *et al.* (2004) pointed out three of 12 Chinese breeds with only one haplotype. The high degree of diversity of the Vietnamese breeds is in agreement with previous reports showing high diversity at the autosomal level analysing microsatellites (Granevitze *et al.*, 2007; Berthouly *et al.*, 2009 and Cuc *et al.*, 2010).

Comparing results of phylogenetic relationship using mtDNA polymorphism and autosomal microsatellites it becomes obvious that the Chinese breeds cluster together and are separated from the Vietnamese local breeds using both genetic marker systems, indicating a clear genetic differentiation between them and the Vietnamese breeds. Although Tieu *et al.* (2008) assumed that the Chinese chickens from NIAS have introgressed into local Vietnamese chickens, our results do not support this hypothesis, except for the Ri and Tau Vang chickens. In contrast to microsatellite analyses, which found that clustering of Vietnamese local breeds has a relationship to their geographical distribution (Cuc *et al.*, 2010), no sub-structuring was found between the Vietnamese local breeds at the mtDNA level. The different results obtained in both types of markers could be due to the different mode of inheritance. Unlike autosomal genetic markers, mtDNA transferred from mother to offspring is not rearranged due to recombination and less affected by gene drift (Johnson *et al.*, 2003). In addition, mtDNA has a lower mutation rate than microsatellite as argued by Feulner *et al.* (2004).

In conclusion, mtDNA analysis done in this study suggests that Vietnamese breeds are of multiple maternal origins, and lineages are distributed across the country. Human immigration from the North to the South in the ancient time could result in the wide distribution of these lineages in both parts of Vietnam. In terms of Chinese breeds, results

showed that the studied breeds are not related to Vietnamese breeds at both genetic levels expect for Ri and Tau Vang.

Acknowledgements

The authors express sincere thanks to the Vietnamese farmers for providing chicken samples, to A. Weigend and A. Flörke, Institute of Farm Animal Genetics at Mariensee, for technical assistance, as well as to Prof. Dr. Clemens B.A. Wollny, Faculty of Life Sciences, University of Applied Sciences Bingen, Germany and Prof. Dr. Hoang Van Tieu, National Institute of Animal Sciences, Hanoi, Vietnam, for project initiative. N.T.K. Cuc had a stipend from German Academic Exchange Service (DAAD), and the project was funded by the German Federal Ministry of Education and Research (BMBF).

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Supplement Table S1. Haplotype names and accession numbers of chicken mtDNA sequences used in this study.

Haplotype name	Accession number	Reference
A1 – A9	GU564361 - GU564369	This study
B1 – B8	GU564370 - GU564377	This study
C	GU564378	This study
D1 – D5	GU564379- GU564383	This study
E1 – E9	GU564384- GU564392	This study
F	GU564393	This study
G1 – G2	GU564394- GU564395	This study
I1 – I2	GU564396- GU564397	This study
Liu_A1	AB114069	Liu <i>et al.</i> (2006) haplotype A1
Liu_B1	AB007744	Liu <i>et al.</i> (2006) haplotype B1
Liu_C1	AB114070	Liu <i>et al.</i> (2006) haplotype C1
Liu_D1	AY588636	Liu <i>et al.</i> (2006) haplotype D1
Liu_E1	AB114076	Liu <i>et al.</i> (2006) haplotype E1
Liu_F1	AF512285	Liu <i>et al.</i> (2006) haplotype F1
Liu_G1	AF512288	Liu <i>et al.</i> (2006) haplotype G1
Liu_H1	D82904	Liu <i>et al.</i> (2006) haplotype H1
Liu_I1	AB009434	Liu <i>et al.</i> (2006) haplotype I1
Oka_D6	AB268535	Oka <i>et al.</i> (2007) haplotype D6
Oka_G1	AB268545	Oka <i>et al.</i> (2007) haplotype G1
Oka_F1	AB268543	Oka <i>et al.</i> (2007) haplotype F1

Supplement Table S2. Distribution of mtDNA D-loop haplotypes in nine Vietnamese local chicken breeds and two Chinese breeds.

Hap.	H'mong	Mia	Ri	Ho	Dong Tao	Te	Choi	Ac	Tau Vang	Luong Phuong	Tam Hoang	Total
A1	6	3	4	3	1	3	3	1	3	2		29
A2			1		2							3
A3				2								2
A4		2										2
A5						1						1
A6			1									1
A7		1										1
A8			1									1
A9									1			1
B1	7	10	5	12	9	10	6	5	4	3	4	75
B2	4	2	1	3	2	3			1			16
B3			1									1
B4					1							1
B5									1			1
B6			1									1
B7		1										1
B8		1										1
C	1		1		1		3		2	1		9
D1			1				7	5	1			14
D2								8				8
D3								2				2
D4									1			1
D5			1									1
E1			2						1	7	11	21
E2									1	3	1	5
E3										3		3
E4											2	2
E5									1		1	2
E6										1		1
E7										1		1
E8											1	1
E9											1	1
F	1				4							5
G1						3						3
G2	1											1
I1									2			1
I2									1			1
Total	20	20	20	20	20	20	19	21	20	21	21	222

Supplement Table S3. Variable sites for 37 mtDNA haplotypes observed in 11 chicken populations.

	1111111111	1111111222	2222222222	2222333333	333
	8155667788	8899999001	1223445566	6789001112	469
	5919492706	8914589683	7263684827	9424673594	398
A1	TCTAGTTTCC	TAAGTCATTC	CTAACCTCC	ATCATTACTA	CCC
A2T
A3T..G	...
A4	..G..C..
A5G.....
A6	C.....
A7T...	...
A8T...
A9	.T.....
B1	.T..A..C..T....T	...
B2	.T..A..C..T....T..T
B3	.T..A..C..T..TT..T
B4	.T..A..C..T....T....
B5	.T..A..C..T....TC.....
B6	.T..A..C..T....TC.....
B7	.T..A..C..T....	..T.....T
B8	.T.....C..T....T
C	.T.....C..	..AC...C.	..G...T.	..G...TC.	...
D1	.T.....C..	...C...CT	..G...CT.
D2	.T.....C..	...C...C.	..G...CT.	T..
D3	.T.....C..	...C...CT	..G...CT.	..G.....	...
D4	.T.....C..	...C...CT	..G...CT.	G..
D5	.T.....C..	...CT..CT	.C.G...T.
E1	.T...C.C..	...C...CTT.T
E2	.TC..C.C..	...C...CTT.T
E3	.T...C.C..	...C...C.T.	..T.....	..T
E4	.T...C.C..	...C...CTT.	..G.....	..T
E5	.T...C.C..	...C...CTT.T
E6	.TC...C..	...C...CTT.T
E7	.TC..C.C..	...C...CTT.TT
E8	.T...C.C..	...C...TT.
E9	.T...C.C..	...C...CTT.
F	.T...C..T	C...CT.CCTTT	C.....	..T
G1	.T..A..C..	...C...CT	...AT.TT	.C.....	..T
G2	.T..A..C..	...C...CT	T...AT.TT	.C.....	..T
I1	.T..A..CT.	.G..CT....	..G...T.C.T.	T..
I2	.T..A..CT.	.G..CT..C.	..GT...T.C.T.	T..

Dots indicate nucleotide positions identical to those of Haplotype A1

Numbers at the top refer to variable sites and correspond to the nucleotide positions of Haplotype A1.

4th CHAPTER

Characterisation of the Vietnamese Chicken Gene Pool compared to Chinese Chicken Breeds

N.T.K. Cuc^{1,2}, H. Simianer¹, H. Eding² and S. Weigend²

¹Department of Animal Sciences, Animal Breeding and Genetics Group, Georg-August-Universität Göttingen, Göttingen, Germany, ²Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt-Mariensee, Germany

Submitted for publication

Summary

*The objective of this study was to characterise Vietnamese local chickens in relation to other Asian breeds from China and wild chickens using molecular markers. The populations collected in Vietnam encompassed nine Vietnamese local breeds and two breeds originating from China but kept in Vietnam. As a reference, data of 14 indigenous breeds from China and three Red Jungle Fowl populations (*Gallus gallus gallus* and *Gallus gallus spadiceus*) were included. All individuals were genotyped at 29 microsatellite loci.*

Using the software package STRUCTURE we found a stable clustering of this diverse gene pool at $K = 5$, in which the Vietnamese breeds formed a homogeneous group separated from the other breeds (except for the Chinese Chahua breed) while the Vietnamese breed Tau Vang and the Chinese breed Tibetan seem to share part of their genome with both local Vietnamese and Chinese breeds. This finding demonstrates that the Vietnamese chicken breeds are genetically different from the Chinese breeds. To estimate the degree of uniqueness of the Vietnamese breeds we analysed the contribution of a single breed to the total diversity of all other breeds and to the diversity of the three Red Jungle fowl populations, respectively. Results revealed that the average contribution of the Vietnamese breeds is higher than that of the reference Chinese breeds to the core set. However, contributions to the set encompassing the Red Jungle fowl populations were lower for Vietnamese populations than for Chinese breeds. This finding suggests that the Vietnamese breeds are closer to Red Jungle Fowl populations than the Chinese breeds.

Keywords: Asian chicken, genetic diversity, phylogenetic structure, microsatellites.

Introduction

Microsatellites are considered as powerful tool for measuring genetic differentiation between closely related livestock breeds (FAO, 1998). Recently, different studies have been published to assess genetic relationships between chicken breeds kept in the different countries using this type of marker. Mwacharo *et al.* (2007) found genetic

subdivision between Kenyan/Uganda and Sudanese/Ethiopian chickens. Studies characterizing European and Asian chicken breeds (Berthouly *et al.*, 2008) or a wide range of chicken breeds and lines from various continents and management systems (Granevitze *et al.*, 2009) showed that relationships between populations were related to their geographical origin and breeding histories.

Vietnam and China are located in Southeast Asia with a common borderline of 1 350 km (GSO, 2008). Human migration from Southern China to Vietnam in the 3rd century B.C. and from the 17th to the 19th century A.C. (Taylor, 1983; Nyiri and Saveliev, 2002) suggests introduction of Chinese chickens to Vietnam. Based on mitochondrial DNA (mtDNA) sequence analysis, Liu *et al.* (2006) showed that one Vietnamese chicken sample clustered in a clade originating from South and Southwest China and/or surrounding areas. Recently, Cuc *et al.* (2010b) found that Vietnamese local chicken breeds presumably originated from Yunnan and adjacent areas in China, South and Southwest China and/or surrounding regions studying mtDNA polymorphism. In contrast, a clear differentiation between the breeds of Vietnamese gene pool and two breeds of Chinese origin kept at National Institute of Animal Sciences (NIAS) was found at both mtDNA and autosomal levels (Cuc *et al.*, 2010a and b). However, this study was based on only two Chinese chicken breeds kept outside of China and may not comprehensively represent the Chinese chicken gene pool. Therefore, it remains still open to what degree chicken breeds of the Vietnamese gene pool separate from Chinese. The aim of the current study was to characterise the differentiation of Vietnamese local breeds from Chinese local chickens including a wider range of indigenous Chinese breeds taken from a previous study. Furthermore, we estimated the contribution of a single Vietnamese breed to the total diversity of all other breeds studied as well as to the diversity of a set of three Red Jungle fowl populations.

Materials and methods

Chicken breeds

The current study used microsatellite genotypes taken from previous studies (Chen *et al.*, 2008 and Cuc *et al.*, 2010a). In both studies, genotyping was done in the same laboratory,

and standard samples were used to adjust for allele scoring. Nine Vietnamese local chicken breeds and two Chinese breeds kept in NIAS as well as 14 Chinese indigenous chicken breeds and three Red Jungle Fowl populations (one *Gallus gallus gallus* and two *Gallus gallus spadiceus* populations) were included. The list of the 28 chicken breeds is shown in Table 1. Detailed description of these populations and sampling is given in the previous reports (Hillel *et al.*, 2003; Chen *et al.*, 2008 and Cuc *et al.*, 2010a). In brief, samples of the Vietnamese breeds were randomly taken from households in eight different districts of four agro-ecological zones from both the southern and northern parts of Vietnam (Cuc *et al.*, 2010a). The two breeds, Tam Hoang and Luong Phuong, from now on named NIAS Chinese breeds in this study, were randomly selected from conservation flocks at the National Institute of Animal Sciences. They were imported from China to Vietnam in 1995 and 2003, respectively. The other 14 Chinese breeds (from now on named reference Chinese breeds) were sampled from conservation flocks kept at the Poultry Institute, Academy of Chinese Agriculture Sciences, Yangzhou and the Centre of Poultry Resources, Anhuni, China (Chen *et al.*, 2008). The three Red Jungle Fowl populations included one population (*Gallus gallus spadiceus*) from China (Chen *et al.*, 2008) and two populations (*Gallus gallus gallus* and *Gallus gallus spadiceus*) from Thailand collected during the EU project AVIANDIV (Hillel *et al.*, 2003).

Table 1. List of 28 chicken breeds of which data taken from previous studies.

Country	Breed	No. of individuals	Reference
Vietnam	H'mong	31	<i>Cuc et al.</i> (2010)
	Dong Tao	32	
	Ho	32	
	Mia	32	
	Ri	32	
	Te	32	
	Choi	33	
	Tau Vang	33	
	Ac	32	
NIAS breeds of Chinese origin	Tam Hoang	32	
	Luong Phuong	32	
China	Xianju	38	<i>Chen et al.</i> (2008)
	Chahua	38	
	Luyuan	34	
	Gushi	40	
	Tibetan	38	
	Baier	34	
	Dagu	35	
	Henan game	33	
	Langshan	40	
	Taihe silkies	40	
	Xiaoshan	40	
	Beijing Fatty	38	
	Huainan partridge	32	
	Wannan three yellow	32	
	<i>Gallus gallus spadiceus</i>	30	
Thailand	<i>Gallus gallus spadiceus</i>	26	<i>Hillel et al.</i> (2003)
	<i>Gallus gallus gallus</i>	30	

Genotyping

Genotyping of 29 microsatellites used in this study was done by PCR as described by Granevitze *et al.* (2007). The RFLPscan software (Scanalytics Division of SSP, Billerica, USA) was applied for electropherogram processing and allele scoring.

Cluster analysis

Population structure was determined using a model-based clustering as implemented in the STRUCTURE software to cluster individuals to a varying number of K clusters (Pritchard *et al.*, 2000). Since we were interested in assessing the separation of the gene pool of Vietnamese local breeds from the Chinese, we ran the analysis only from K = 2 to K = 6. The analysis of STRUCTURE was done as described by Granevitze *et al.* (2009) with 100 independent runs for each K value. The SIMCOEFF software (Rosenberg *et al.*, 2002) was used to compare repeated runs, and solutions with over 95% similarity were considered identical. In addition, a method proposed by Evanno *et al.* (2005) to detect the optimal number of clusters was applied.

Phylogenetic network

Marker estimated kinships (MEK) between and within populations were calculated according to Eding and Meuwissen (2001). Calculation of MEK based on similarity indices used the Weighted Drift Similarity model (Oliehoek *et al.*, 2006). The kinship matrix was transformed into a distance matrix and used as an input file for the SPLITSTREE v. 4.6 (Hudson and Bryant, 2006) to construct a phylogenetic network.

Genetic differentiation between breeds and private alleles

The average F_{ST} estimates (Weir and Cockerham, 1984) between breeds within groups of the Vietnamese, NIAS and reference Chinese and Red Jungle Fowl populations, respectively, were computed using FSTAT v. 2.9.3.2 (Goudet, 2001). The numbers of private alleles of these groups were calculated using the Microsatellite Toolkit v. 3.1.1 (Park, 2001).

Optimal core set contribution and 'safe set' analysis

The relative importance of each Vietnamese and NIAS Chinese breed was assessed by calculating the optimal contributions to a core set. A core set is a mixture of populations composed such that the genetic overlap within the core set is minimized. Kinship is defined as the probability that two alleles randomly sampled from a population are identical by descent. Therefore, if the average kinship in a set of breeds is minimized, the fraction of alleles descending from the same ancestor will be minimized as well, eliminating much of the genetic overlap between populations. Hence, the contribution of each breed to the core set is adjusted such that the mean kinship is minimized. Moreover, since the fraction of founder additive genetic variance is proportional to $(1 - f)$, where f is the mean kinship, it follows that minimizing the mean kinship within the core set maximizes the additive genetic variance present in the core set.

Optimal contributions were derived according to Eding *et al.* (2002) from a matrix containing the mean kinship within (diagonal) and between (off-diagonal) populations. These 'optimal contributions' provide a ranking of breeds according to the amount of genetic diversity they contribute to the total diversity present in a set of populations. This enables the evaluation of the relative importance of each breed to the conservation of genetic diversity. For more details, see Eding *et al.* (2002).

To assess the degree of uniqueness of the Vietnamese breeds relative to Red Jungle fowl populations, a 'safe set' analysis was performed (Eding *et al.*, 2002). In this type of analysis a baseline set (the 'safe set') is defined. This 'safe set' contains populations that are either considered safe from extinction or have a priori been chosen for conservation. Populations not in the safe set are included in this set each in turn. For these alternative (safe + i) sets the contributions are optimized and the conserved genetic diversity is calculated. The difference in conserved genetic diversity (defined as $1 - f$) between the safe set and the (safe + i) set is taken as the genetic diversity the one non-safe population adds to the safe set. This added diversity is used as an indicator of the relative importance of a population in relation to the safe set. For the sake of the analysis we assumed three Red Jungle fowl populations are safe. A set of these three populations was defined as the 'safe set'. We compared the diversity lost by only retaining this existing 'safe set' to the 'safe set plus one' of the other breed. Both the total genetic diversity of the existing set

plus one Vietnamese breed - $Div(S + i)$ - and the diversity added by a single Vietnamese breed to the 'safe set' - $d(i)$ - were calculated.

Results

Cluster analysis

Results of STRUCTURE analysis for a clustering the set of 28 breeds into two ($K = 2$) and five ($K = 5$) clusters, respectively, are given in Figure 1. At $K=2$, we found highly repeatable clustering with 100% identical solutions at threshold of 0.95 of similarity coefficients between runs. At this level of clustering the Vietnamese breeds grouped together with the Red Jungle Fowl and were separated from the NIAS and reference Chinese breeds (except for the Chinese Chahua). Applying the method of Evanno (Evanno *et al.*, 2005) suggested an optimal clustering at $K = 5$. At $K = 5$, Vietnamese breeds clustered together with the Chinese Chahua breed forming one cluster, and the Red Jungle Fowl populations made up their own cluster while the NIAS and the other reference Chinese breeds formed the other remaining three clusters. The two NIAS Chinese breeds clustered together with the heavy body weight type of breeds of the reference Chinese breeds whilst the Vietnamese Tau Vang and Chinese Tibetan breeds appeared as admixture populations between Vietnamese and Chinese gene pools.

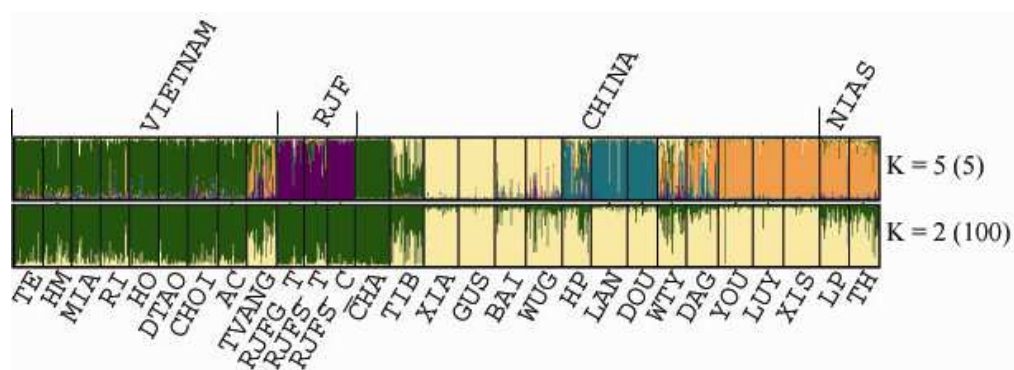


Figure 1. Clustering of 28 populations under study. Number in parenthesis is the number of identical solutions of 100 repeats at 95% threshold.

Key: HM = H'mong; DTAO = Dong Tao; TVANG = Tau Vang; RJFSG_T = Thailand *Gallus gallus gallus*; RJFS_T = Thailand *Gallus gallus spadiceus*; RJFS_C = Chinese *Gallus gallus spadiceus*; CHA = Chahua; TIB = Tibetan; XIA = Xianju; GUS = Gushi; BAI = Baier; WUG = Taihe Silkies; HP Huainan Partridge; LAN = Langshan; DOU = Henan game; WTY = Wannan Three-yellow; DAG = Dagu; YOU = Beijing Fatty; LUY = Luyuan; XIS = Xiaoshan; LP = Luong Phuong; TH = Tam Hoang.

Phylogenetic network

The phylogenetic network constructed from the kinship distances of 28 breeds is shown in Figure 2. The Vietnamese breeds clustered closely with the Red Jungle fowl populations and the Chinese Chahua breed, but were separated from clusters of the other reference and NIAS Chinese breeds, with the Vietnamese Tau Vang and Chinese Tibetan breeds in the middle. The Red Jungle fowl populations showed longer branches than the Vietnamese breeds.

had 34 private alleles. From these 34 private alleles, 25 alleles occurred at a frequency of less than one percent, while the remaining varied from 1.21 to 4.51%. In comparison, almost all (15/16) private alleles found in the reference Chinese breeds had a frequency of less than one percent and only one allele occurred at a frequency of 2.94%.

Table 2. The average F_{ST} estimate between breeds and unique alleles in the groups of the Vietnamese, reference Chinese, Red Jungle Fowl and NIAS Chinese breeds.

Population group	Number of breeds	$F_{ST} \pm SD$	Number of Unique alleles
Vietnamese	9	0.0511 ± 0.0298	34
NIAS Chinese	2	0.062	1
Reference Chinese	14	0.1506 ± 0.0501	16
Red Jungle Fowl	3	0.1718 ± 0.0514	8

Optimal core set contribution and 'safe set' analysis

Contributions of the Vietnamese breeds to the core set and to the 'safe set' (consisting of a set of Red Jungle fowl populations) are shown in Table 3. The total conserved genetic diversity in the core set was 0.970, while total diversity of the Red Jungle Fowl existing set was 0.920. This result suggests that 97% and 92%, respectively, of the genetic variance in the founder population is conserved in the complete set of breeds and the set of Red Jungle Fowl populations, respectively. The average contribution of the Vietnamese breeds is higher than that of the reference Chinese breeds to the core set. However, contributions to the 'safe set' were lower for Vietnamese populations than for Chinese breeds.

Within the group of Vietnamese breeds, the highest contribution to the core set was observed in the Ac breed while the lowest was found in the Ho breed. The diversity added to the 'safe set' by individual Vietnamese populations was highest for the Tau Vang and lowest for the Ho breed.

Table 3. Genetic contribution and added genetic diversity of 11 populations under study to total diversity of all other breeds and to a safe set consisting of three Red Jungle Fowl populations.

Breed		Contribution	Red Jungle fowl	
			Div (S+i)	d(i)*1000
	Safe set		0.920	
Vietnamese	Ac	0.0555	0.936	16.296
	Choi	0.0433	0.935	14.951
	H'Mong	0.0424	0.937	17.076
	Tau Vang	0.0416	0.936	17.721
	Mia	0.0362	0.936	16.426
	Te	0.0268	0.935	14.834
	Ri	0.0198	0.935	15.342
	Dong Tao	0.0171	0.934	14.751
	Ho	0.0156	0.935	14.374
		Mean	0.0331	0.935
	SD	0.0140	0.001	1.169
NIAS Chinese	Luong Phuong	0.0602	0.939	18.574
	Tam Hoang	0.212	0.936	15.790
	Mean	0.1361	0.936	17.182
	SD	0.1073	0.002	1.967
Reference Chinese	Mean	0.0285	0.937	10.645
		0.0162	0.002	1.532

Key: NIAS = National Institute of Animal Science.

Div (S+i) = total diversity of the safe set plus one population, d(i) = diversity added by breed i to safe set S.

Discussion

A separation of the Vietnamese breeds from the two NIAS Chinese breeds found in this study indicated that the two NIAS Chinese breeds do not share their genome with the Vietnamese breeds. This finding strengthens an argument from a previous study at the mtDNA level (Cuc *et al.*, 2010b) that there is no gene introgression of these two NIAS Chinese breeds into local Vietnamese chickens. Furthermore, clustering the two NIAS Chinese breeds together with the reference Chinese breeds indicates that these two breeds are part of the Chinese gene pool.

The results of STRUCTURE and phylogenetic relationship analyses indicated a clear genetic difference between the Vietnamese and reference Chinese breeds (except for the Chahua breed kept in Yunnan). The genetic distinction of these populations suggests genetic isolation for a long time and limitation of gene flow between breeds with an exception of the Chahua breed. Gene flow might be hindered as populations of both gene pools are kept in two different countries (i.e., Vietnam and China). Clustering of the Chahua breed with the Vietnamese breeds might be due to the close neighborhood of the Yunnan province. Human migration from Southern Chinese people (i.e., Yunnan) to Vietnam (Taylor, 1983; Nyiri and Saveliev, 2002) may have led to interbreeding between breeds of the Yunnan region and the Vietnamese chicken breeds. Based on mtDNA analysis, Cuc *et al.* (2010b) found that Vietnamese domestic chickens may have originated from Yunnan, South and Southwest China and/or surrounding areas.

The Vietnamese breeds clustered into one homogeneous group while the Chinese breeds grouped into three clusters. In addition, average F_{ST} between Vietnamese breeds was lower than between Chinese breeds (Table 2). This shows that differences between Chinese breeds are more pronounced than between Vietnamese breeds which might be attributed to lower geographic distance and higher gene flow within the group of Vietnamese breeds.

Although the number of Vietnamese breeds studied was lower than the number of reference Chinese breeds (9 breeds vs. 14 breeds, respectively), the number of private alleles observed in the Vietnamese group was about twice as high as in the reference Chinese breeds. The large number of private alleles observed in this study supports the

finding of Granevitze *et al.* (2007) who found that the Vietnamese H'mong breed carried the highest number of private alleles compared to a wide range of chicken populations originating from various continents and management systems.

Optimised contributions of a single breed show that Vietnamese breeds contributed more to total genetic diversity than reference Chinese breeds. The correlation between expected heterozygosity and core set contribution was significantly positive ($P < 0.05$, Figure S1) Hence high core set contributions might be attributed to the high genetic diversity within Vietnamese breeds compared to reference Chinese breeds (the heterozygosity values of 64% vs. 57%, respectively).

Detailed analyses of genetic contribution of each Vietnamese chicken breed to the total core set and the set of Red Jungle Fowl populations show that the Ho and Dong Tao breeds scored consistently low. In contrast, the ranking of other breeds differed between the core set and the 'safe set' analyses. Especially, the difference in ranking of the Ac breed in the core set and 'safe set' analyses indicated that this breed adds considerable genetic diversity to the total set while it shared much diversity with Red Jungle Fowl populations. This suggests that the Ac breed could comprise unique genetic features compared to three groups of the Vietnamese, NIAS breeds, and Chinese breeds and could be an important source of variation which contributes substantially to future breeding programs.

Clustering of Vietnamese breeds together with the Red Jungle fowl populations at low K values in the STRUCTURE analysis indicates that the Vietnamese breeds are more similar to the Red Jungle fowl populations than the NIAS and reference Chinese breeds. However, at higher K values ($K = 5$) the Red Jungle fowl populations split from the Vietnamese breeds and formed their own cluster. Similar phylogenetic relationships were displayed in the network analysis, in which the Red Jungle fowl grouped together with Vietnamese breeds but showed longer branches than the Vietnamese breeds. This indicated that the Red Jungle fowl populations are different from Vietnamese breeds which is inconsistent with previous reports of Berthouly *et al.* (2009) who showed an admixture between Vietnamese scavenging domestic chickens in the Ha Giang province of Vietnam and wild chickens sampled from different geographic origins (i.e., Thailand and Vietnam, but latter was maintained in a French zoological park). This difference

could be due to the different areas and populations studied. Our study included neither chickens from the Ha Giang province nor Vietnamese Red Jungle Fowl populations. On the other hand, the Vietnamese chicken gene pool shared more genetic diversity with the Red Jungle Fowl populations than Chinese breeds suggesting a rather close relationship between the Vietnamese chickens and Red Jungle Fowl populations. This has also been shown in the phylogenetic network and STRUCTURE analyses, respectively, in which the Vietnamese breeds clustered together with Red Jungle Fowl populations while reference Chinese breeds (except for the Chahua breed) were separated. Del Hoyo *et al.* (2001) found that Southwest Yunnan, China and Vietnam are part of the distribution of *Gallus gallus spadiceus* and *Gallus gallus jabouillei*. This can be used to explain why the Vietnamese breeds are closer to the Red Jungle Fowl populations than the Chinese breeds, since almost all of them (13/14) are not kept in Southwest Yunnan, China. Further studies involving these populations should be taken into consideration.

In summary, results from this study indicate that Vietnamese local chicken breeds are genetically isolated from Chinese indigenous chicken breeds. The results reveal that the Vietnamese breeds are more similar to Red Jungle Fowl populations than the reference Chinese breeds.

Acknowledgements

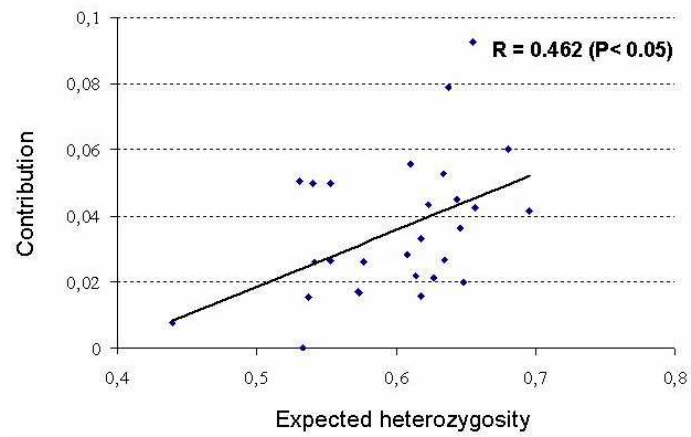
The authors express sincere thanks to A. Weigend and A. Flörke, Institute of Farm Animal Genetics at Mariensee for technical assistance. We thank the Vietnamese farmers for providing chicken blood samples. N.T.K. Cuc had a stipend from German Academic Exchange Service (DAAD). The project was funded by the German Federal Ministry of Education and Research (BMBF).

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Supplementary Figure 2. Relationship between expected heterozygosities and contributions of 28 breeds.

5th CHAPTER

Conservation priorities and optimum allocation of conservation funds for Vietnamese local chicken breeds

N. T. K. Cuc^{1,2,3}, S. Weigend², H. V. Tieu³ and H. Simianer¹

¹Department of Animal Sciences, Animal Breeding and Genetics Group, Georg-August-Universität Göttingen, Göttingen, Germany, ²Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt-Mariensee, Germany, ³National Institute of Animal Sciences, Hanoi, Vietnam.

Submitted for publication

Summary

The objectives of this study were to estimate conservation potential of Vietnamese local breeds and to investigate optimal allocation of conservation funds to maximize genetic diversity conserved between these breeds. Twenty-nine microsatellites were genotyped in 353 individuals from nine Vietnamese local chicken breeds and two chicken breeds of Chinese origin. The Vietnamese chicken breeds were sampled from the northern and southern parts of Vietnam while the two Chinese breeds have been kept as conservation flocks at the National Institute of Animal Sciences, Hanoi. The Weitzman approach was used to assess alternative strategies for conserving genetic diversity between breeds. Three different models, which reflect the range of possible functions in typical conservation situations, were applied. An average extinction probability of 48.5% was estimated for all Vietnamese chicken breeds. The highest conservation potential was found in the Te, Dong Tao and Ac chicken breeds, whereas the lowest corresponding values were observed in the Ri and Mia chicken breeds. The conservation funds were mainly allocated to the same three breeds (Te, Dong Tao and Ac) under all three models. This study suggests that conservation potential of the Vietnamese chicken breeds varies considerably. The three breeds Te, Dong Tao and Ac with the highest conservation potential should be the prime candidates to be allocated conservation funds if the conservation budgets are limited. Population priorities for allocation of conservation funds in this case do not depend on the cost model used, so that a similar share of allocation under all three models is given to the Te and Dong Tao chicken breeds each.

Keyword: Weitzman approach, conservation priority, Vietnamese local chickens, allocation of resources

Introduction

The erosion and loss of animal genetic resources would compromise the flexibility of future breeding measures and enhance rural development (FAO, 2007a). Poultry genetic resources are considered to be the most endangered and under-conserved, and strategic approaches to conservation at the national level need to be developed and implemented (Hoffmann, 2009).

Weitzman's (1992) diversity concept is widely used as a formal approach to rational decision making in livestock conservation. Weitzman's theory was applied in studies on various farm animal species, such as chicken (Pinent *et al.*, 2005), pig (Laval *et al.*, 2000 and Fabuel *et al.*, 2004), cattle (Thaon d'Arnoldi *et al.*, 1998; Canon *et al.*, 2001; Simianer, 2002; Reist-Marti *et al.*, 2003, Simianer *et al.*, 2003; Tapio *et al.*, 2006; Zerabruk *et al.*, 2007 and Zander *et al.*, 2009), horse (Thirstrup *et al.*, 2008) and dog (Leroy *et al.*, 2009). The approach combines genetic diversity assessed at the molecular level and extinction probabilities estimated by socio-economic factors to derive conservation priorities of breeds based on their conservation potential. Using conservation potentials for prioritizing breeds is very efficient in selection of breeds for conservation, when the objective of the conservation plan is to maximize the genetic diversity conserved (Reist-Marti *et al.*, 2003; Simianer *et al.*, 2003 and Zerabruk *et al.*, 2007).

Although the conservation potential is considered as a good indicator for conservation decision, it does not give information on how to allocate the conservation budget to maximize the conserved diversity. Simianer *et al.* (2003) proposed an algorithm to find the optimum allocation of a given quantity of conservation resources to maximize the expected diversity. This algorithm links conservation expenditures to reduction in extinction probability and a range of possible functions in the typical conservation situation are suggested.

In Vietnam, besides one local chicken breed (Van Phu breed) reported to be extinct, 11 breeds have been recorded in the World Watch list (<http://dad.fao.org>), of which six breeds have been decreasing in numbers and one breed is critically endangered (National Institute of Animal Sciences, 2000). During recent years, some exotic chicken breeds were imported to Vietnam due to their high performance. This could result in the disappearance of Vietnamese local chicken breeds (Tieu *et al.*, 2008). National programs on conservation of the Vietnamese farm animal genetic resources have been initiated since 1990 in order to prevent the extinction of local animal breeds. However, conservation decisions were made mainly based on population size information and population trend of the breeds. Other indicators affecting extinction probability of the breeds and the contribution of the breeds to total genetic diversity have not been taken into account, and allocation of conservation funds might not ensure to prevent the loss of

national animal genetic resources in the long-term (Tieu *et al.*, 2008). The objectives of this study were to estimate the conservation potential of Vietnamese local chicken breeds and to investigate optimal allocation of conservation funds to minimize loss of genetic diversity between these breeds.

Materials and Methods

Breeds

Nine Vietnamese chicken breeds and two breeds of Chinese origin were studied (Table 1). The Vietnamese Ri and Tau Vang breeds are the most popular dual-purpose, while the other Vietnamese chicken breeds were kept for different purposes (Su *et al.*, 2004), such as cultural reasons (Ho, Choi and Mia breeds), the unique feature of short and thick legs (Dong Tao breed), dwarfism (Te breed) and traditional tonics (Ac and H'mong breeds). Samples of the nine Vietnamese breeds were collected in eight districts of four agro ecological zones located in both the northern and southern parts of Vietnam. On average, 32 individuals were randomly taken for each breed by sampling one male and one female per household. Two breeds of Chinese origin, Tam Hoang and Luong Phuong, were used as reference populations. They were imported to Vietnam in 1995 and 2003, respectively, and kept as conservation flocks at the National Institute of Animal Sciences (NIAS). Blood samples from 32 Chinese individuals (16 males and 16 females per breed) were collected and genotyped at 29 microsatellite loci, as described by Granevitze *et al.* (2007).

Questionnaire administration

Data on general socio-economic factors were collected from households who provided chicken blood samples. A structured questionnaire was administered to 15 households per breed, except for the Te chicken breed for which only 4 households could be surveyed due to their rareness. Information on change of the total population size over the last five years, the risk of indiscriminate crossing, disease control and production system of the breed were captured during the interviews. Furthermore, information on the farmer's sources of income, their participation in the farmers union, the perception of the farmers regarding the value of the breed such as special traits and socio-cultural importance were

recorded. Data on estimated total population size, geographical distribution of the breed and conservation schemes were completed by Prof. Tieu (The head of the National Animal Genetic Resources Conservation Programs, one of the authors) based on his knowledge of the breeds.

Table 1. Description of populations sampled in Vietnam.

Breed	Agro ecological zone	Study area	No. of interviewed households	No. of blood samples
H'mong	Northwest	Mai Son, Son La	15	31
Dong Tao	Red River Delta	Khoai Chau, Hung Yen	15	32
Ho		Thuan Thanh, Bac Ninh	15	32
Mia		Duong Lam, Ha Tay	15	32
Ri		Hoai Duc, Ha Tay	15	32
Te		Ba Vi, Ha Tay	4	8
		NIAS		24
Choi	South Central Coast	Ninh Hoa, Khanh Hoa	15	33
Tau Vang	Mekong Delta	Tan an, Long An	15	33
Ac			15	32
Tam Hoang		NIAS		32
Luong Phuong				32
Total			124	353

Current diversity, maximum-likelihood tree and genetic diversity contribution

The total Weitzman diversity (D) of a set S , $D(S)$ is identical to the ordinates of all nodes in the maximum likelihood tree if the tree drawn horizontally. The sum of the branch lengths are reduced by the total height of the tree to give the diversity (Thaon d'Arnoldi *et al.*, 1998). It is defined recursively as (Eding and Bennewitz, 2007)

$$D(S) = \max_{i \in S} [D(S_{i \notin S}) + d(i, S_{i \notin S})]$$

where $D(S)$ is the diversity function of any set S of breeds, $S_{i \notin S}$ stands for set S without breed i and $d(i, S_{i \notin S})$ for the distance between breed i and the set without i .

Reynolds genetic distance (Reynolds *et al.*, 1983) was estimated by PHYLIP 3.57c software (Felsenstein, 1993). Weitzman's (1992) recursive algorithm was used to construct maximum-likelihood tree. The maximum-likelihood tree is the evolution scheme which maximizes the probability that every element of the set S exists at the current time (Thaon d'Arnoldi *et al.*, 1998). Maximum-likelihood trees were illustrated using TreeView 32 software (Page, 1996).

The contribution of an element is proportional to the reduction in tree length caused by its removal from the group. The contribution c_i of breed i to the diversity of the set S was calculated as (Reist-Marti *et al.*, 2003)

$$c_i = D(S) - D(S \setminus i)$$

where $D(S)$ is represented as the sum of the ordinates of all nodes of the tree if the tree is drawn horizontally; $D(S \setminus i)$ is the diversity of the set without breed i .

Extinction probability

In this study, we mainly used the scoring method suggested by Reist-Marti *et al.* (2003). Our scheme encompasses 11 variables which we believe to be the most relevant factors affecting the survival and extinction probability of Vietnamese chicken breeds. The scheme is representative of different aspects, such as population description, environmental impact and present value of the breed. The population description was determined by two variables: total population size (POS) and the change of total population size over the last five years (CHA). The environmental impacts were reflected by seven variables: distribution of the breed (DIS), risk of indiscriminate crossing (CRO), disease control (DIS), production system (PRO), organization of farmers (ORG), establishment of a conservation scheme (CON) and economic development (ECO). The present "value" of a breed was displayed by the variables of special traits (SPE) and socio-cultural importance (CUL). The range of values was assigned based on the estimated magnitude of the variable. The weight of variables is shown in Table 2. Extinction probability of the two Chinese breeds was set to zero because it is assumed that these breeds are safe in China.

The three variables, population size, established conservation scheme and changing of population size over time were scored by the highest values. The variables of population size and established conservation scheme were weighted between 0 for no impact on risk, and 0.3 for high impact on risk. The variable of changing of population size over time was scaled from -0.1 to 0.2. These variables were weighed highest because current population size and population growth trends are the most important factors determining the risk status. Where populations are small, there is a greater likelihood for a rapid extinction. When population is above a certain population size, the risk can be regarded as small. The more rapidly a population builds up to reach the critical size, the less it is exposed to the risk of extinction. Besides these two variables, further consideration is given to active conservation program (Scherf, 2000).

The effect of geographic distribution of the breed was indicated by scoring between 0 for no impact on risk and 0.2 for high impact on risk. The latter pertained to the situation that a limited geographical distribution of the population is linked to a high risk of complete extinction when the population is exposed to threats such as disease epidemics, natural disasters, or inappropriate management (FAO, 2007b and Woolliams, 2004). The Te chicken breed with unidentified distribution was considered as spreading within region.

The three variables, economic development, production system and disease control were considered in this study because the number of chickens kept in the Vietnamese households has a relationship to the level of their income based on chickens (Epprecht, 2005), and high chick mortality often is due to mishandling, outbreaks and predation (Burgos *et al.*, 2008). In addition, poultry diseases are considered as a large threat to traditional poultry production in Vietnam (FAO, 2009). Therefore, these variables are included and assigned values between 0 for no impact on risk and 0.1 for high impact on risk. A similar scoring is also made for the four remaining variables based on Reist-Marti *et al.* (2003).

Table 2. Variables and criteria for the estimation of extinction probability of Vietnamese chicken breeds.

Variable (abbreviation)	Criterion and value
Total population size (POS)	0.3 < 1 000 0.2 = 1 001 to 10 000 0.1 = 10 001 to 100 000 0.0 > 100 000
Changing of total population over the last 5 years (CHA)	0.2 = decreasing 0.0 = stable -0.1 = increasing missing value = 0.2
Distribution of the breed (DIS)	0.2 = localized (in one province) 0.1 = spread within region 0.0 = widespread within country
Degree or risk of indiscriminate crossing (CRO)	0.1 = high 0.00 = marginal
Disease control (DC)	0.1 = no 0.0 = yes
Production system (PS)	0.1 = scavenging without fence 0.0 = scavenging with fence or semi-intensive
Organization of farmer (ORG)	0.1 = no 0.0 = yes
Established conservation scheme (CON)	0.3 = none 0.1 = in-situ 0.0 = ex-situ
Economic development (ECO)	0.1 = income from others 0.05 = income from chicken and others 0.0 = income from chicken only
Special use (SPE)	0.1 = none 0.0 = yes
Socio-cultural importance (CUL)	0.1 = none 0.05 = some socio-cultural 0.0 = yes

The estimates of total population size and information on distribution as well as conservation schemes of the breed were retrieved from Prof. Hoang Van Tieu. Values of the other variables were calculated from the household surveyed. Value of the variable of changing of total population size was calculated as follows:

$$z_{ia} = \left(\frac{N_{1ia}}{T_i} * (-0.1) \right) + \left(\frac{N_{2ia}}{T_i} * 0.0 \right) + \left(\frac{N_{3ia}}{T_i} * 0.2 \right)$$

where z_{ia} is the value of the variable of changing of total population size of breed i . T_i is a total number of the households surveyed per breed i . N_{1ia} , N_{2ia} and N_{3ia} are the numbers of the households of breed i responsible for increasing, stable and decreasing population, respectively.

Values of the two variables, economic development and socio-cultural importance, were estimated by the following formula:

$$z_{ia} = \left(\frac{N_{1ia}}{T_i} * 0 \right) + \left(\frac{N_{2ia}}{T_i} * 0.05 \right) + \left(\frac{N_{3ia}}{T_i} * 0.1 \right)$$

where z_{ia} is the value of variable a of breed i . T_i is a total number of the households surveyed per breed i . N_{1ia} , N_{2ia} and N_{3ia} are the number of the households of breed i responsible for no impact, medium impact and high impact of variable, respectively.

Values of the five variables, degree of indiscriminate crossing, disease control, production system, organization of the farmer and special use, were calculated as follows:

$$z_{ia} = \left(\frac{H_{1ia}}{T_i} * 0 \right) + \left(\frac{H_{2ia}}{T_i} * 0.1 \right)$$

where z_{ia} is the value of variable a of breed i . T_i is a total number of the households surveyed per breed i . N_{1ia} and N_{2ia} are the number of the households of breed i responsible for no impact and impact, respectively.

The extinction probability of breed i , z_i , was computed as the sum of the values of the 11 variables. If a breed was considered to be completely safe, the value of the extinction probability is equal to zero and this value will be 1 if a breed is certain to go extinct. The sum of eleven variables was rescaled to a value from 0.1 to 0.9 as follows.

$$z_i = \frac{0.8}{1.7} * \sum_{a=1}^{11} z_{ia} + 0.147$$

Expected future diversity, marginal diversity and conservation potential

The expected diversity of the set after a given time t accounts for the extinction probability z_i for every breed i of the set. The probability that at the end of the given time, situation K will arise, where a particular subset of breeds exists and the complementary subset is extinct, is (Simianer *et al.*, 2003)

$$P(K) = \prod_i (k_i + (-1)^{k_i} z_i)$$

where: $i = 1, \dots, N$; $k_i = 1$ if breed i is still existing and $k_i = 0$ if i is extinct in the set K .

From the above, 2^N different combinations of present and extinct breeds are possible, for which the respective probabilities can be calculated as described. When D_K is diversity of the set of breeds not extinct, i.e., with $k_i = 1$, the expected diversity at the end of the time t is estimated as

$$E[D(S)] = \sum_K P(K) D_K$$

The variance of expected diversity can be computed as

$$Var[D(S)] = \sum_K P(K) [D_K^2 - (E[D(S)])^2]$$

The marginal diversity D_i' of a breed indicates the change of the expected diversity in the whole population when the extinction probability of breed i is increased by one unit. While the position of the breeds in the tree and the extinction probability of the neighbouring breeds in the tree play important roles for the value of the marginal

diversity of a breed, the extinction probability of breed i itself is not taken into account. The marginal diversity of the breed i (Simianer *et al.*, 2003) is

$$D'_i = \frac{\partial E[D(S)]}{\partial z_i}$$

Conservation potential describes the possible increase in expected diversity if an endangered breed is made completely safe. Conservation potential CP_i of breed i will be calculated as follows (Simianer *et al.*, 2003):

$$CP_i = -D'_i \times z_i$$

Optimum allocation of conservation funds

To maximize the expected amount of between breed diversity conserved, a hypothetical conservation fund was allocated among nine Vietnamese breeds under the assumptions of three different models as suggested by Simianer *et al.* (2003). These models reflect different approaches to breed conservation.

Model A assumes that the effect of a conservation program is to increase the effective population size by an additive increment which is proportional to the amount of money spent per animal in this population. In this scenario, the height of the premium per animal plays an important role in the farmer decision to keep animals.

Model B assumes a multiplicative effect of a conservation program on the effective population size which is proportional to the amount of money spent per individual in the population. The objective of this model is to increase or to keep flock sizes constant. A practical approach is to raise one male offspring of each sire and one female offspring of each dam for replacement. Thus, expenditures are obviously relative to the population size and, with more animals, more extension specialists are required.

Model C assumes a multiplicative effect on the effective population size proportional to the funds spent per population. Under this model, the effect of a conservation scheme on effective population size is assumed to be multiplicative with a factor that is only depending on the amount of money spent in any population, irrespective of its effective size. In this model, costs of information channels are independent on the population size

and number of farmers due to an assumption of breeding organization established. Therefore, the cost for extension specialists is neglected.

These three models span a wide range of possible conservation policies, and so the application of all three models will identify the relevant range of sensible allocation patterns. For more details of the models, see Simianer *et al.* (2003).

Results

Current diversity, maximum-likelihood tree and genetic diversity contribution

The actual diversity for the whole group was 0.531. The maximum-likelihood tree showed that the Northwest chicken breed H'mong and the mixed chicken breed Te grouped together into one cluster. Four Red River Delta chicken breeds (Mia, Ri, Ho and Dong Tao) formed a close cluster with an outlier of the South Central Coast chicken breed Choi while the Mekong Delta chicken breed Ac had its own branch. The Mekong Delta chicken breed Tau Vang clustered together with the two Chinese chicken breeds into a separate branch. The three chicken breeds, Ac, Choi and Dong Tao showed the highest genetic contributions (Figure 1).

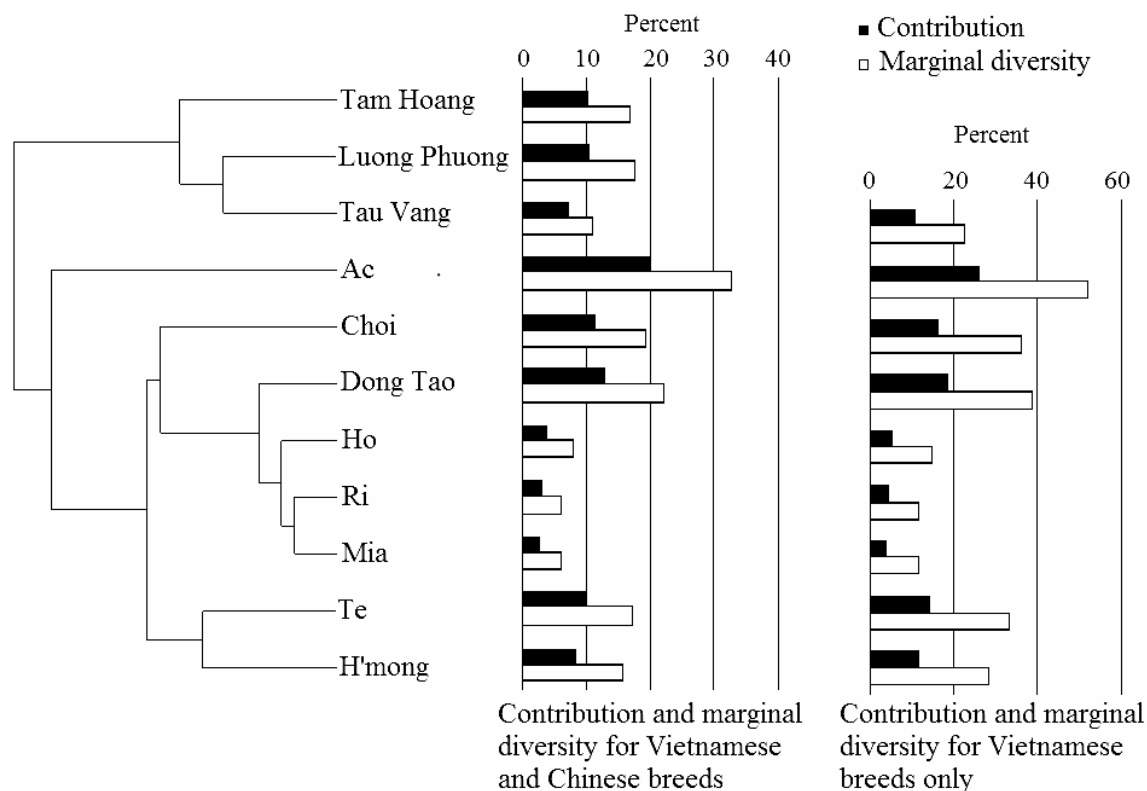


Figure 1. Marginal genetic diversity and genetic diversity contribution of breeds for nine Vietnamese local breeds only and considering two Chinese breeds.

Extinction Probabilities

All calculated extinction probabilities are shown in Table 3. Over all breeds, the average estimated extinction probability is 0.485 ± 0.121 . The highest estimated extinction probability of 0.735 was observed in the Te chicken breed, followed by the Ho (0.593) and Dong Tao chicken breeds (0.545), while the lowest corresponding values were found in the Choi, Ri, Ac, Mia and H'mong chicken breeds, ranging from 0.373 to 0.409. Statistically significant positive correlations existed between the five pairs of variables: CHA and POS, PRO and DIC, CUL and CRO, CUL and ORG. The variable POS and CHA were significantly and positively correlated with the extinction probability (Table 4).

Table 3. Estimated extinction probabilities (z) of Vietnamese local chicken breeds.

Breed	Variable*											z
	POS	CHA	DIS	CRO	DIC	PRO	ORG	CON	ECO	SPE	CUL	
Ac	0.100	-0.093	0.000	0.000	0.000	0.000	0.100	0.300	0.043	0.000	0.100	0.406
Choi	0.200	-0.020	0.000	0.000	0.000	0.000	0.000	0.100	0.070	0.100	0.030	0.373
Dong Tao	0.300	-0.007	0.100	0.000	0.013	0.000	0.100	0.100	0.067	0.100	0.073	0.545
Ho	0.300	0.167	0.200	0.000	0.000	0.000	0.000	0.100	0.080	0.100	0.000	0.593
Ri	0.000	-0.033	0.100	0.100	0.000	0.000	0.100	0.000	0.063	0.100	0.100	0.396
Mia	0.200	-0.007	0.100	0.000	0.000	0.000	0.000	0.100	0.050	0.100	0.053	0.409
Te	0.300	0.20	0.100	0.100	0.100	0.100	0.100	0.000	0.050	0.100	0.100	0.735
H'mong	0.000	0.000	0.100	0.000	0.100	0.100	0.100	0.000	0.100	0.000	0.050	0.406
Tau Vang	0.000	-0.093	0.100	0.100	0.000	0.000	0.100	0.300	0.047	0.100	0.100	0.502
Mean	0.156	0.013	0.089	0.033	0.024	0.022	0.067	0.111	0.063	0.078	0.067	0.485
SD	0.133	0.103	0.060	0.050	0.043	0.044	0.050	0.116	0.018	0.044	0.037	0.121

* See Table 2 for definitions of variable abbreviation

Table 4. Correlation and significance among the extinction probability (z) of Vietnamese local breeds and variable* used for its estimation.

	POS	CHA	DIS	CRO	DIC	PRO	ORG	CON	ECO	SPE	CUL
CHA	0.66 ^b										
DIS	0.24	0.57									
CRO	-0.31	0.12	0.14								
DIC	0.02	0.50	0.11	0.17							
PRO	-0.02	0.50	0.10	0.19	0.99 ^c						
ORG	-0.44	-0.18	-0.14	0.50	0.41	0.38					
CON	-0.21	-0.56	-0.34	-0.07	-0.55	-0.54	0.07				
ECO	-0.06	0.26	0.34	-0.41	0.37	0.36	-0.14	-0.56			
SPE	0.45	0.30	0.37	0.38	-0.34	-0.36	-0.38	-0.19	-0.25		
CUL	-0.39	-0.28	-0.20	0.65 ^b	0.16	0.15	0.84 ^a	0.22	-0.60	-0.15	
z	0.61 ^b	0.80 ^a	0.527	0.37	0.43	0.41	0.18	-0.18	-0.14	0.36	0.17

* See Table 2 for definitions of variable abbreviation

a,b,c: P<0.05; 0.01; 0.001

Expected future diversity, marginal diversity and conservation potential

The expected diversity after 50 years would be 0.241 ± 0.107 . The marginal diversities of the breeds are shown in Figure 1. In both cases the highest conservation potentials were observed in the Te, Dong Tao and Ac chicken breeds while the lowest corresponding estimates were found for the Ri and Mia chicken breeds (Figure 2).

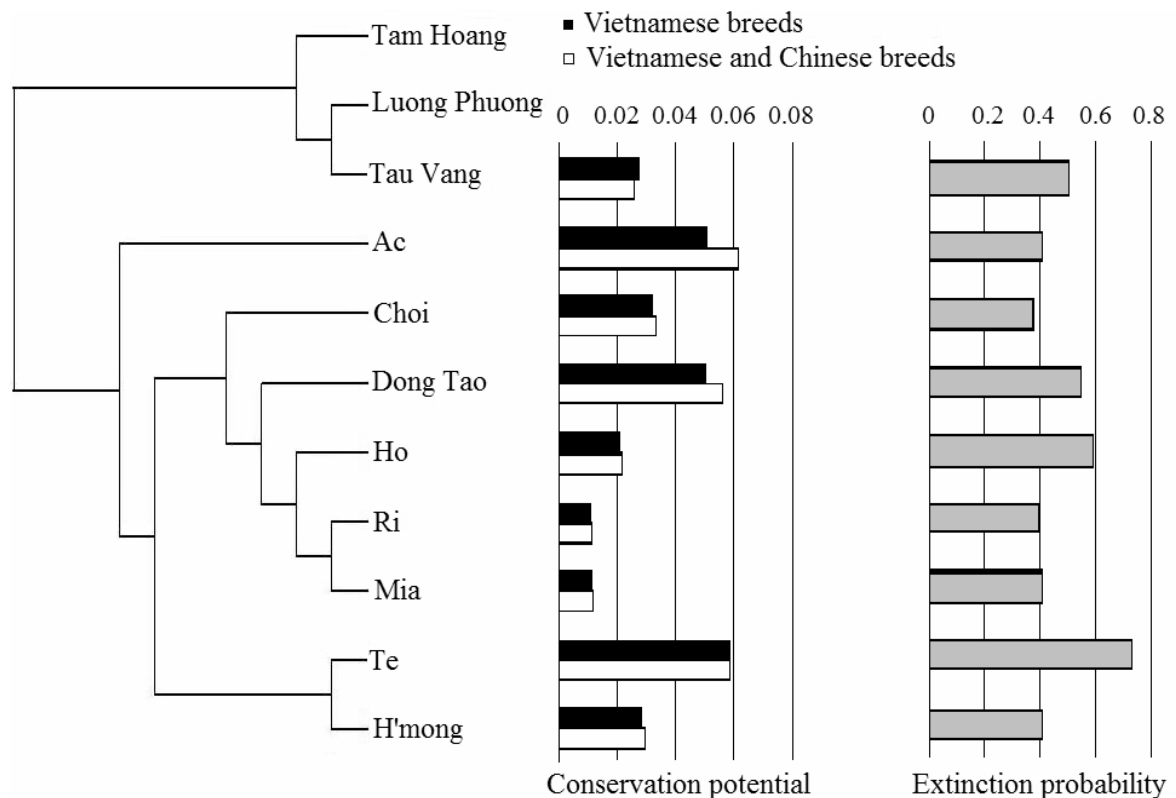


Figure 2. Maximum-likelihood tree of Vietnamese and Chinese breeds, extinction probabilities of nine Vietnamese breeds and conservation potential for each breed for nine Vietnamese breeds.

Optimum allocation of conservation funds

Optimal allocation of resources under the three assumed models is given in Figure 3. Under all three models, the largest share of the available funds is allocated to conserve the Te chicken breed, followed by Dong Tao and Ac. Model B and C allocate funds only to three breed (Te, Dong Tao and Ac). Although model A shared funds to five chicken breeds, the majority (90%) of funds was also distributed to these three breeds. A similar share of allocation under all three models was given to the Te and Dong Tao chicken breeds each while the Ho and Tau Vang chicken breed received a relatively minor share under the model A and no share under model B and C.

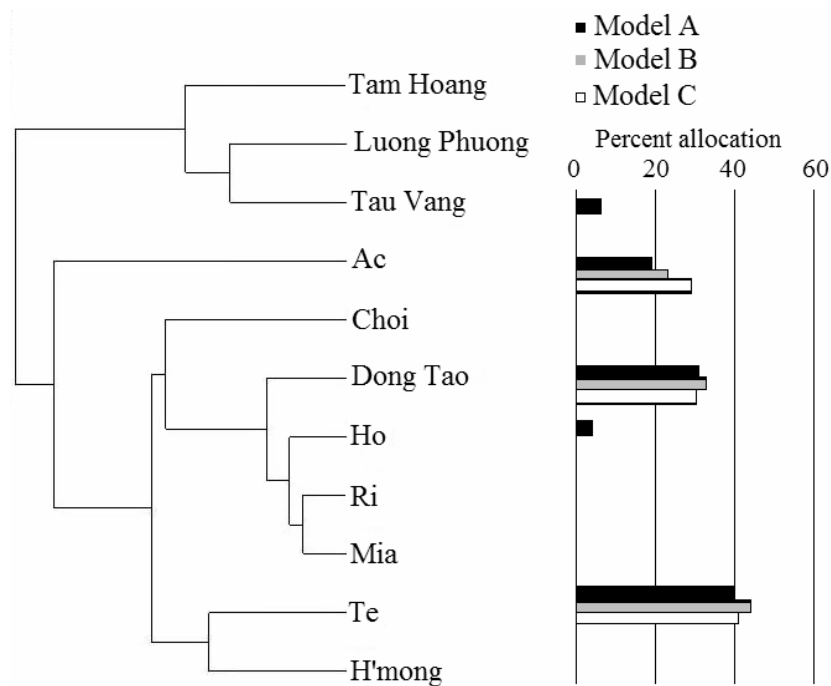


Figure 3. Maximum-likelihood tree and optimum allocation of resources for nine Vietnamese breeds under the three models considered.

Discussion

Maximum-likelihood tree and genetic diversity contribution

Given genetic diversity between breeds, the maximum-likelihood tree supports previous findings based on STRUCTURE analysis (Cuc *et al.*, 2010) who reported that genetic relationships of the Vietnamese local chickens are related to their geographical distribution. Small geographical distances between the Red River Delta areas, where the chicken breeds under study are kept, could enable gene flow between them, resulting in the Red River Delta chicken breeds grouped together. In contrast, gene flow is hampered due to a strong geographical isolation between the Mekong Delta breed Ac and the Northern and the South Central Coast breeds. This might have led to the separate branch of the Ac breed. In addition, distinct characteristics (i.e., small body size, white feather and black skin and bone) of the Ac breed could inhibit its gene introgression into other breeds, causing reproductive isolation. This finding shows a high contribution of the Ac breed to total diversity. Therefore, it could be an important source of variation which contributes substantially to a long term conservation plan of national chicken genetic

resources and future breeding strategies. Clustering of Tau Vang breed from the Mekong Delta together with the Chinese chicken breeds is in agreement with the Chinese origin of the Tau Vang chickens (Linh, 2005). The two Chinese chicken breeds being grouped separately from the Vietnamese chicken breeds supports the argument that the Chinese chicken breeds kept in NIAS harbour a separate gene pool different from the Vietnamese chicken breeds (Cuc *et al.*, 2010). Therefore, the two Chinese chicken breeds should not be considered as part of the Vietnamese chicken gene pool to be conserved.

A ranking of genetic contribution of the Vietnamese chicken breeds also confirms the results displayed in the maximum–likelihood tree, in which breeds clustering closely together in the maximum–likelihood tree were found to have the lowest contribution while breeds grouped separately showed highest contribution. However, Reist-Marti *et al.* (2003) argued that genetic contribution is not the only basis for decisions about conservation.

Extinction Probabilities

The majority of variables in this study were calculated according to the survey data from households in the field. Therefore, our scheme estimates the threat of extinction of a breed in its environmental context, takes the most important factors into account separately and thus shows the specific impact of different conservation efforts on particular risk factors. Extinction probability of the breeds estimated in this study is in reasonable agreement with their known survival states. The average extinction probability of 48.5% estimated in the Vietnamese local chicken breeds, with four of nine breeds having values higher than 50%, is corresponding with the FAO's estimation for the risk status of the world's chicken breeds with about 33% considered endangered to critical breeds and another 40% with unknown risk status (FAO, 2007b). The high extinction probabilities of three chicken breeds (Te, Dong Tao and Ho) are mainly attributed to their small population sizes, the unfavourable estimates of population trend (Te and Ho breeds) and the localized distribution (Ho breed). This finding indicated that these three breeds are at great risk of being wiped out by natural disasters, disease, or inappropriate management.

The significant positive correlation between socio-cultural importance (CUL) and risk of indiscriminate crossing (CRO) might be explained by the fact that Vietnamese local chicken breeds are considered as a part of the cultural heritage of a certain area or people. This cultural heritage has been inherited from previous generations, and it should be passed to the next generations, i.e., almost all households said that their parents kept the same chicken breed and this breed will be recommended to their children (data not shown). In addition, these farmers are relatively conservative. They want to keep their traditions and habits and often live in isolated communities. Therefore, the households in the community often kept only one local chicken breed of the region (FAO, 2009). A significant positive correlation between CUL and farmer organizations (ORG) found in this study indicates the efforts of the local farmer community to conserve the cultural values of the breed in the region. Although CUL was not significantly correlated with extinction probability, all households stated that they kept Ho chickens mainly for socio-cultural reasons. Hence, the cultural value of the breeds must be taken into account in the conservation decisions.

Expected future diversity, marginal diversity and conservation potential

The expected future diversity of the whole group was about half the current diversity, which reflects a high average extinction probability of 48.5%, with one-third of the chicken breeds having values in the range of 50–74%. Our results suggest that if no conservation efforts are made and all other factors remain the same, half of the genetic diversity of the Vietnamese local chickens will be lost within the next 30–50 years.

Bennewitz *et al.* (2007) indicated three different selection strategies in animal conservation decision. They are the maximum-risk-strategy, the maximum-diversity-strategy and the maximum-utility-strategy. The maximum-risk-strategy uses basically the degree of endangerment of a breed as the sole selection criteria. The maximum-diversity-strategy is a combination of extinction probability and information on the phylogenetic structure to estimate the conservation potential of a breed. The later strategy is an extension of the maximum-diversity-strategy which is to maintain certain features or characteristics of breeds due to their present or potential future value.

The application of maximum-risk-strategy in this study resulted in the highest conservation priority for three chicken breeds: Te, Dong Tao and Ho breeds. With this strategy, maximum genetic diversity could not be maintained due to the Ho breed having minor genetic contribution and low marginal diversity. In contrast, when the selection of a breed for conservation considers information on phylogenetic structure only, three chicken breeds (Ac, Choi and Dong Tao) contributed most (Figure 1) although the Ac and Choi have a low degree of endangerment. Therefore, using either the maximum-risk-strategy or information on phylogenetic structure only are both sub-optimal. When the maximum-diversity-strategy was applied, three chicken breeds (Te, Dong Tao and Ac) represented the highest estimated conservation potential. The high conservation potentials of these three breeds are mainly attributed to high extinction probability estimated in the Te and Dong Tao breeds and the elevated value of the marginal genetic diversity observed in the Ac breed.

Considering the maximum-utility-strategy, when the objective of a conservation plan is to maintain certain features or characteristics of the Vietnamese breeds (i.e., unique features of short and thick legs, dwarfism as well as beautiful appearance), conservation of such features is simply achieved by putting effort into conservation of the single breed (i.e., Dong Tao, Te and Ho breeds, respectively) which carry those defined features. If these breeds are maintained with the defined feature, its marginal utility will be very high. The gene pool will completely lose the feature if these breeds carrying the feature go extinct.

Optimum allocation of conservation funds

The importance of an optimal allocation of budget was indicated by Simianer (2002) who found that with optimum allocation only about 50% of the resources are required for the same conservation effect that can be achieved when conservation funds either are shared equally over all breeds or are focused on the most endangered breeds only. In this study, model A assumes that a fix amount of money per year pays a premium to the owner of chickens, balancing some of the economic inferiority of those chickens. Model B presumes that a basic strategy for maintaining diversity in small population is to keep family size constant. In this case, the costs are proportional to the population size. Model C reflects the same strategy as Model B but the costs are independent of the population size. The results of this study shows that population priority for optimum allocation of

conservation funds for Vietnamese breeds is almost independent of the assumed allocation patterns. Under all three models, three breeds (Te, Dong Tao and Ac) are prime candidates for conservation expenditure which might be attributed to their highest conservation potential. This also shows that the prioritisation approach is robust with respect to the assumed model of allocation. Since the definition of a detailed cost model for conservation measures is highly complex (Reist-Marti *et al.*, 2006), the demonstrated robustness of decision making towards a misspecification of the cost model appears reassuring. This finding is in agreement with the study of Simianer *et al.* (2003) who found that in general the highest amount of funds should be allocated to the breeds that show a large conservation potential.

The importance of a particular breed for the conservation of genetic diversity and allocation of conservation funding depends on the strategy chosen. The Weitzman approach applied in this study did not take into account migration between breeds or within breed diversity (Eding and Meuwissen, 2001; Caballero and Toro, 2002 and Tapio *et al.*, 2006). Although reducing extinction probability in the Weitzman approach by increasing the effective population size is also a measure to prevent a loss of the within breed genetic diversity (Simianer *et al.*, 2003), and a study of Pinent *et al.* (2005) in European chicken breeds showed that breeds with very small population size were not favoured by the Weitzman's approach, the shortcoming of this approach should be borne in mind. The contribution to the Weitzman diversity suggests keeping separate breeds to maintain allelic diversity within species, ensuring access to maximum adaptive potential of species (Simianer, 2005 and Tapio *et al.*, 2006). The Vietnamese local breeds were kept in different agro-ecological zones across the country with various local production systems. The Vietnamese farmers keep their local breed only in the region (FAO, 2009). In addition, the analysis of the genetic structure of these breeds indicated an absence of gene flow between breeds kept in the different agro-ecological zones (Cuc *et al.*, 2010). Therefore, the Weitzman approach with the assumption of no crossbreeding is considered to be a reasonable fundament for decision-making on conservation strategy for the Vietnamese local chicken breeds.

The results of this study indicate that the conservation potential of the Vietnamese local breeds varies considerably. The between genetic diversity of Vietnamese local chicken breeds can be maintained by conserving and prioritizing the breeds with highest

conservation potential. Population priorities for conservation allocation of the Vietnamese breeds do not depend on which cost model was used. Conservation funds should be primarily spent on three breeds (Te, Dong Tao and Ac) with the highest conservation potential if the conservation budgets are limited. The breed Te and Dong Tao each receive a similar share under all three models. However, conservation decisions also have to take into account other factors, such as genetic diversity within breed, socio-cultural role, special features, current or future economic and scientific values of the breed. The choice of breeds and the amount of money allocated to each breed should also consider the cost effectiveness of conservation activities in different breeds, the effects of the optimal conservation programs and the cost of conservation program as indicated by Reist-Marti *et al.* (2006).

Acknowledgements

The authors express sincere thanks to the Vietnamese farmers for providing information and chicken samples, to A. Weigend and A. Flörke, Institute of Farm Animal Genetics at Mariensee for technical assistance, and to Prof. Dr. Clemens B.A. Wollny, Faculty of Life Sciences, University of Applied Sciences Bingen, Germany for the project initiative. N.T.K. Cuc had a stipend from German Academic Exchange Service (DAAD), and the project was funded by the German Federal Ministry of Education and Research (BMBF).

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6th CHAPTER

General discussion

General discussion

The overall goal of this study was to characterize the diversity of the Vietnamese local chicken breeds and to identify population priorities for conservation. The specific aims were 1) to assess and explain the population genetic structure of the Vietnamese local chicken breeds, 2) to characterise Vietnamese local chickens in relation to other Asian breeds from China and wild chickens, 3) to estimate conservation potentials for conservation priorities, and 4) to define an optimal allocation of limited conservation funds to Vietnamese chicken breeds. Vietnamese local chickens play an important role in small householder farms but their existence is under threat due to low performance of local breeds, farmers' preferences for exotic breeds and the lack of adequate conservation strategies (Tieu *et al.*, 2008). The current national conservation programs rely on definition of breeds based on phenotypic characteristics only. In addition, conservation decisions neglect genetic contribution of the breeds to total genetic diversity and allocations of conservation funds might not guarantee the avoidance of loss of genetic variability in the most efficient way (Tieu *et al.*, 2008).

As described in Chapter 1, in Vietnam, as in other developing countries (Kitalyi and Mayer, 1998; Coplan and Alders, 2005; Alders and Pym, 2010), local chickens are owned by families at the village level. Production of local chickens is feasible at village level, in which low investments are required. Although chickens require low inputs, chicken production makes an important contribution to livability of local populations through being an important protein source and contributing to poverty alleviation, reduction of gender inequalities and maintenance of cultural traditions (Epprecht, 2005; Cuc *et al.*, 2006 and Burgos *et al.*, 2007). In addition, the genetic potential of indigenous chicken could be a reservoir of genetic variation. Local chickens might contain genes and alleles pertinent to their adaptation to particular environmental conditions and local breeding goals (Horst, 1989) which are not present in the highly selected commercial lines.

Genetic diversity within and between the Vietnamese local chicken breeds at the autosomal level was assessed in Chapter 2. Microsatellites are highly polymorphic, co-dominant DNA markers which have been widely used in population genetics since they give much information for a limited number of loci (Tixier-Boichard *et al.*, 2009) while single nucleotide polymorphism (SNPs) are a more recent development (Twito *et al.*,

2007 and Muir *et al.*, 2008). Genetic diversity estimations using microsatellite markers have been shown to provide a reliable assessment of genetic diversity (Hillel *et al.*, 2007; Berthouly *et al.*, 2008; Granevitze *et al.*, 2007 and 2009). A set of 29 microsatellite markers used in this study is based on a recommendation of FAO (2004). These markers were also used to assess genetic diversity of chicken populations in previous studies (Cuc *et al.*, 2006; Hillel *et al.*, 2007; Muchadeyi *et al.*, 2007; Chen *et al.*, 2008; Granevitze *et al.*, 2007; 2009 and Bodzsar *et al.*, 2009). Using the same markers provides an insight into genetic variability of the Vietnamese chicken breeds in comparison to those of the other chicken breeds found at the global level.

The mean allele number (6.09 ± 0.67) and expected heterozygosity estimate (0.634 ± 0.034) per population in this study is lower than estimated in Zimbabwean chickens (6.32 ± 0.24 and 0.651 ± 0.007 , respectively, Muchadeyi *et al.*, 2007) but higher than reports on Chinese (4.61 ± 0.77 and 0.568 ± 0.054 , respectively, Chen *et al.*, 2008) and Hungarian chickens (3.42 ± 0.48 and 0.518 ± 0.039 , respectively, Bodzsar *et al.*, 2009) as well as commercial lines (3.58 ± 0.60 and 0.52 ± 0.077 , respectively, Granevitze *et al.*, 2007). The high numbers of alleles and expected heterozygosity of the Vietnamese breeds compared to Chinese, Hungarian and purebred populations were expected, because Vietnamese breeds were field populations without any management, while Chinese breeds were collected from conservation flocks (Chen *et al.*, 2008), and Hungarian as well as purebred populations were selected for special traits and/or were of small population size (Bodzsar *et al.*, 2009 and Granevitze *et al.* 2007, respectively). High level of genetic diversity was observed in Vietnamese chickens because these field flocks are freely roaming during scavenging. This fact might result in the migration of chickens from one flock to the neighbor's one. The migration results in a continuous gene flow between these flocks, conserving a high number of alleles and the heterozygosity within a breed. This applies only at village level. The low numbers of alleles and expected heterozygosity of the Vietnamese village chickens compared to Zimbabwean village chickens might be due to differences in population structures observed in these two countries. No population sub-structuring was found between Zimbabwean populations (Muchadeyi *et al.*, 2007) whereas the Vietnamese breeds showed a clear sub-structuring in this study. As a consequence, the level of gene flow between the Zimbabwean populations is higher than between the Vietnamese breeds.

The separation between the Vietnamese breeds in three geographically defined clusters, namely the northern part, the South Central Coast as well as the Mekong Delta in the STRUCTURE analysis may reflect gene pools with different genetic features due to adaptation to the specific environments and limited gene flow as a result of geographical isolation between them. Taking into account that geographical isolation of breeds contributes to genetic differentiation (Tixier-Boichard *et al.* 2009), the genetic sub-structure may indicate that the Vietnamese chickens in different regions may comprise specialized populations. The large geographic distances and the difference in topography, soil pattern, climate, farming systems, social and economic factors between these regions have played an important role in the structuring of the breeds. A high level of genetic differentiation between breeds of different areas found in this study suggests that the Vietnamese local breeds might have evolved a unique adaptation to their agricultural production system and agro-ecological environment. They may present a diverse gene pool that could comprise unique genetic features. However, microsatellites used in this study do not infer about adaptative genetic diversity of the Vietnamese local chickens. Further studies on assessing genetic polymorphism in candidate genes and large scale sequence studies should be, therefore, taken into consideration.

The results of this study showed that the two Chinese chicken breeds kept in the National Institute of Animal Sciences (NIAS Chinese breeds) were genetically distinct from the Vietnamese chicken gene pool. Although they were imported to Vietnam in 1995 (Tam Hoang) and 2003 (Luong Phuong), these two breeds have not been introgressed into the Vietnamese local chicken gene pool with an exception of the Tau Vang breed which showed an admixture between Vietnamese and NIAS Chinese gene pools in the STRUCTURE analysis. Genetic distinctiveness of the two NIAS Chinese breeds from the Vietnamese local breeds found in this study suggests that the two NIAS Chinese breeds are not Vietnamese genetic resources and are, therefore, not of high priority for conservation in Vietnam.

Microsatellites are autosomal markers. Loci are either unlinked and alleles of different loci are inherited independently from each other or, if loci are linked, they might be rearranged in the process of the recombination, If loci are not linked to genes under selection their changes in allele frequencies are mainly affected by genetic drift (Randi *et al.*, 2004). Mitochondrial DNA (mtDNA) sequences are maternally inherited and tend to

be maintained amid populations interbreeding. They are less affected by genetic drift (Feulner *et al.*, 2004). A share of maternal lineages between recent populations and populations dating further back in time can be analysed due to the absence of recombination in mtDNA. The mtDNA sequences are capable of providing information on genetic structure which is inherited on the maternal path and can be used to explain the observed genetic structures in particular when combined with other nuclear markers such as microsatellites.

The analysis of the mtDNA sequences in Chapter 3 showed that the Vietnamese breeds can be assigned to eight distinct maternal lineages based on a skeleton reflected suggested regions of domestication in chickens. This skeleton plays an important role because it is constructed from clades which indicate apparent geographic affiliation for domestication events. The skeleton was based on the most frequent haplotypes of the nine clades of Liu's network (Liu *et al.*, 2006) and three additional clades (D, G and F) of Oka *et al.* (2007). In eight distinct maternal lineages contributed to Vietnamese breeds found, three maternal lineages which presumably originated from Yunnan, South and Southwest China and/or surrounding areas (i.e., Vietnam, Burma, Thailand and India) have the most common contribution to the Vietnamese local chickens. This finding supports the report of (Taylor, 1983) who argued that Vietnamese people have originated from China due to human immigration from southern China into Vietnam in the 3rd century B.C. The result of this study is in agreement with the earlier reports (Liu *et al.*, 2006; FAO, 2007; Oka *et al.*, 2007 and Kanginakudru *et al.*, 2008) which indicated that domestication in chicken occurred in China, Southeast Asia and Indian subcontinent.

The nine Vietnamese breeds studied shared the two major haplotypes, resulting in no population sub-structuring. The hypothesis can be built that the Vietnamese breeds, which were distributed across the country, share two common maternal lineages leading to lack of divergence between them. This is supported by a report on human migration from the North to the South of Vietnam (Nyiri and Saveliev, 2002). Although no population sub-structuring between the nine Vietnamese breeds was found at the mtDNA level, they are diverse in haplotypes. This haplotype diversity corresponded well to the high degree of variability found at the microsatellite level (Chapter 2). High haplotype diversity within Vietnamese breeds might be attributed to a high number ($n = 8$) of distinct maternal lineages contributing to Vietnamese breeds. Haplotype diversity of the Vietnamese breeds

(0.78 ± 0.10) is higher than that of other chickens in the studies of Muchadeyi *et al.* (2008) and Liu *et al.* (2004). Haplotype diversities of Zimbabwean chickens and purebred lines are 0.67 ± 0.05 and 0.60 ± 0.20 , respectively (Muchadeyi *et al.*, 2008), and three of 12 Chinese breeds show only one haplotype in the study of Liu *et al.* (2004).

The lack of a detectable population sub-structuring between Vietnamese local breeds at the mtDNA level is contrary to the findings at the microsatellite level where clustering of Vietnamese local breeds was correlated with geographical distance. This difference may be due to the different nature of the two marker systems reflecting different evolutionary forces and time scales. Microsatellite markers describe the consequences of recent genetic isolation between populations (Randi *et al.*, 2004) while mtDNA is informative for assessing long-term maternal lineages (Feulner *et al.*, 2004). Therefore, this finding suggests that the Vietnamese breeds which are recently genetically isolated share various maternal lineages. In addition, a lower mutation rate in mtDNA sequences than in microsatellites, as argued by Feulner *et al.* (2004), could be used to explain the different findings found in this study. The observation in this study is in agreement with the study of Johnson *et al.* (2003) who indicated that recent fragmentation and isolation of greater prairie-chicken populations in Midwestern North America has had a stronger effect on microsatellite than on mtDNA population structure.

The results of mtDNA analysis also showed that the two NIAS Chinese breeds clustered into a distinct clade separate from the Vietnamese breeds. This suggests that the NIAS Chinese chickens originated from a different maternal gene pool than the Vietnamese chickens. Both mtDNA and microsatellite findings strengthen the argument that the two gene pools most likely have been isolated from each other for a long time. A clear differentiation between the Vietnamese gene pool and the two NIAS Chinese breeds found at both genetic levels (Chapter 2 and 3) raises questions about divergence between the Vietnamese and the Chinese chicken gene pool and the level of representativeness of the two NIAS Chinese for the Chinese chicken gene pool. Therefore, a wider range of indigenous Chinese breeds taken from a previous study of Chen *et al.* (2008) was included to answer these questions as indicated in Chapter 4.

The result of Chapter 4 indicates that the two NIAS Chinese breeds are part of the Chinese gene pool. The findings in this chapter provide additional evidence that the

Vietnamese gene pool is genetically different from the Chinese gene pool even when a wider range of breed is considered. This indicates that the Vietnamese and Chinese chickens are isolated from each other and a restricted gene flow occurred between them. The only exception is the breed Chahua which has originated from the Yunnan region. The clustering of this breed together with Vietnamese breeds strengthens the argument (Chapter 3) that suggested Yunnan, South and Southwest China and/or surrounding areas (i.e., Vietnam, Burma, Thailand and India) as origins of the Vietnamese breeds.

Compared to the reference Chinese breeds, the Vietnamese breeds showed a low estimate of genetic diversity between breeds ($F_{ST} = 0.051 \pm 0.030$ vs. 0.151 ± 0.050 , respectively) but a high average value of expected heterozygosity (0.634 ± 0.034 vs. 0.568 ± 0.054 , respectively). The core set contribution significantly correlated with the expected heterozygosity found in this study. This explains why the Vietnamese breeds contributed more genetic diversity to the total diversity of a core set than the reference Chinese breeds, even if genetic diversity between Vietnamese breeds is lower than the reference Chinese breeds. The high contribution of the Vietnamese breeds to the total set agrees well with the number of private alleles observed in the Vietnamese group. This number was about twice as much as that observed in the reference Chinese breeds although the sample size of Vietnamese breeds is lower than the Chinese breeds (nine breeds vs. 14 breeds, respectively).

Furthermore, the Vietnamese breeds clustered together with the Red Jungle fowl populations. This finding indicates that, compared to Chinese gene pool, genetic diversity of the Vietnamese chicken gene pool is more overlapping with that of the Red Jungle Fowl populations. Several previous studies (Darwin, 1868; Fumihito *et al.*, 1996 and Hillel *et al.*, 2003) stated the hypothesis that the Red Jungle fowl was the main ancestor of the domesticated chicken. Based on the assumption that the more distant populations are the more different they will be in terms of their genetic features, the results of this study suggest that the Vietnamese gene pool might carry more genetic features of the ancestor of domesticated chicken than the NIAS and reference Chinese. Therefore the Vietnamese chicken breeds could be considered as an important source of chicken genetic diversity which needs to be conserved to prevent the loss of valuable genetic variability and unique characteristics.

The expected diversity of all Vietnamese breeds and the conservation potential of each breed were estimated in Chapter 5. The expected diversity reflects the amount of diversity to be expected after 30 to 50 years if no conservation efforts are made. Conservation potential of a breed reflects how much the expected diversity can be increased if this breed is made completely safe. These estimations were based on the approach suggested by Weitzman (1992 and 1993) and illustrated by Thaon d'Arnoldi *et al.* (1998). In addition, optimal allocation of conservation funds to minimize loss of genetic diversity between these breeds was investigated. Three cost models reflect the conservation effect per unit conservation fund spent in terms of reduced extinction probability. They span a wide range of possible conservation policies as indicated by Simianer *et al.* (2003). Given the information on the budget spent for conservation in all breeds is available, one can derive the optimum allocation of the same amount of resources, in the sense of optimising the objective function.

A difference of Weitzman's approach compared to the core set method is that the Weitzman approach uses only genetic distances, so it does not take into account within breed diversity. The other difference is its iterative character. The approach designates the contribution to total diversity for each breed in dependence of all other breeds and calculates its conservation potential not only based on the gain of saved diversity per amount spent on a specific breed but also on extinction probability affected by socio-economic factors.

The 11 parameters to determine extinction probabilities in this study relied on the report of Reist-Marti *et al.* (2003). They are mainly based on the effective population size, the population trend and conservation schemes. This method was also used to estimate the extinction probability of North Ethiopian cattle breeds (Zerabruk *et al.*, 2007) and was adapted for evaluating the degree of endangerment of German chicken breeds (Pinent *et al.*, 2005) and Borana cattle breeds in East African (Zander *et al.*, 2009). The scheme used in this study yields reasonable estimates of extinction probabilities for the Vietnamese breeds, which were significantly influenced by the population size and its recent change. The extinction probability over all Vietnamese breeds was lower than German breeds (49% vs. 59%, respectively) but in the same range with the FAO's estimation for the world's chicken breeds which indicated that about 33% of the breeds are considered endangered to critical and another 40% of the breeds with unknown risk

status (FAO, 2007). The high extinction probability of Vietnamese breeds compared to German breeds can be explained by the fact that chicken production in Vietnam is mainly based on local breeds (Desvaux *et al.*, 2008) while Germany is one of the European countries that have the most highly specialized livestock industries, in which production is dominated by a small number of commercial breeds (FAO, 2007), and local breeds are mainly maintained by hobby keepers and fancy breeders, resulting in their high risk status.

The maximum-likelihood tree supports the results of Chapter 2, that genetic relationships of Vietnamese local chickens are related to their geographical distribution. A separate clustering of the two NIAS Chinese chicken breeds from Vietnamese gene pool is in agreement with the results in Chapter 2 and Chapter 3. Therefore, it is clear that these two breeds should not be considered as part of Vietnamese chicken gene pool to be conserved.

The expected genetic diversity of Vietnamese breeds after 30 – 50 years was about a half of the current diversity, and the coefficient of variation was about 10%. This finding implies that conservation efforts are needed to avoid the erosion of genetic diversity between Vietnamese breeds. A clear definition of objectives is crucial for all conservation activities (Simianer, 2005 and FAO, 2007). The first question which has to be answered in all conservation programs is: which breeds should be prioritised conservation. An identification of priority breeds for conservation depends on the objectives (i.e., maximization of conserved genetic variation or conservation of genetic diversity between breeds) of conservation programs. When the objective of the conservation measures is to conserve as much genetic diversity between breeds as possible, then the Weitzman method with the assumption of no crossbreeding should be used (Simianer, 2005). The Vietnamese local breeds showed an absence of gene flow between breeds kept in different agro-ecological zones in the STRUCTURE analysis (Chapter 2). Therefore, the Weitzman approach used in this study is considered to be a reasonable fundament for decision-making on conservation strategy for the Vietnamese local chicken breeds.

A high conservation potential can either result from a high degree of endangerment, or from a high marginal diversity. The marginal diversity reflects the breed's phylogenetic position. It also indicates whether closely related breeds are safe from extinction, but is independent of the breed's own extinction probability (Simianer *et al.*, 2003). In this study, The Ri and Mia breeds have the lowest conservation potential due to low estimates

(0.397 and 0.409, respectively) of their extinction probabilities and their closest relationship displayed in the maximum-likelihood tree. The latter can be explained by the fact that these two breeds are collected from two districts of the same provinces (Hoai Duc and Duong Lam district, Ha Tay province, respectively). A small geographical distance (26.2 km) between these two districts might enable a high level of gene flow between the Ri and Mia breeds, resulting in a high genetic similarity. In contrast, the highest conservation potentials observed in the Te, Dong Tao and Ac chicken breeds are attributed by high extinction probabilities estimated in the Te and Dong Tao breeds and the distinction of Ac breed from the other Vietnamese breeds represented in the maximum-likelihood tree. The results suggest that the optimum conservation strategies to maximise genetic diversity between Vietnamese breeds should prioritize the breeds with the highest conservation potential, rather than the most endangered breeds. Therefore, three breeds (Te, Dong Tao and Ac) should be given priorities to be conserved.

FAO (2007) declared that efficient conservation programmes should use available funds in an optimal way. The following question in all conservation programs is: what share of the total conservation budget should be allocated to each of the chosen breeds when the budgets are limited. To conserve as much genetic diversity between Vietnamese chicken breeds as possible, population priorities for conservation allocation of the Vietnamese breeds do not depend on which cost model is used. Conservation funds should be allocated to three breeds (Te, Dong Tao and Ac) under all three models. A similar share of allocation under all three models is given to the Te and Dong Tao chicken breeds each. These findings confirm that the allocation priority should be given to those breeds with the highest conservation potential and not to the most endangered breeds.

Conclusions

From this study several conclusions can be drawn:

- i. At the autosomal level, the Vietnamese local chicken breeds from different agro-ecological zones represent genetically distinct populations. The northern breeds are clearly separated from the South Central Coast breed and from the Mekong Delta breed.

- ii. The Vietnamese local chicken breeds are highly polymorphic and originated from eight maternal lineages. These lineages are present across the country.
- iii. Two chicken breeds of Chinese origin, Tam Hoang and Luong Phuong, kept in the National Institute of Animal Sciences are genetically distinct from the Vietnamese local breeds.
- iv. The Vietnamese chicken breeds are genetically separated from the Chinese chicken gene pool.
- v. The Vietnamese chicken breeds are more closely related to wild Red Jungle fowl populations than the Chinese breeds.
- vi. The expected future diversity of the Vietnamese chicken breeds after 30 to 50 years is only a half of the current diversity and average extinction probability of the breeds was about 49%.
- vii. Conservation potential of the Vietnamese chicken breeds varies considerably.
- viii. The three breeds Te, Dong Tao and Ac with the highest conservation potential should be the prime candidates to which conservation funds should be allocated if budgets are limited.

Implications and recommendations

The high genetic diversity between the Vietnamese chicken breeds and their high contribution to the total diversity of a given set of populations implies their importance as a genetic resource for future use. For breeding purposes, the immigration of genes represented in the different Vietnamese breeds in the various agro-ecological zones is very effective in re-establishing diversity to catch accumulated inbreeding. However, further research is needed whether Vietnamese breeds have experienced different selection pressures because microsatellite markers do not give information on population differences in relevant production traits. An analysis of polymorphisms at candidate genes and large scale high throughput genotyping or sequencing studies would add more

information on the population structures and breed histories of Vietnamese chicken breeds.

The Vietnamese breeds shared more genetic diversity of the Red Jungle Fowl populations than the reference Chinese breeds as discussed in Chapter 4. The Vietnamese breeds clustered together with the Chahua breed which has exchanged gene with Red Jungle Fowls (Liu *et al.*, 1996). In addition, Berthouly *et al.* (2009) showed that there is gene flow between the Vietnamese chickens of the Ha Giang province with Red Jungle fowl populations. Therefore, Vietnamese Red Jungle fowl populations should be analysed using the same markers to assess whether gene flow between Red Jungle fowl populations and Vietnamese breeds used in this study occurred. This further study would add further information on genetic relationship between the Vietnamese breeds and Red Jungle fowl populations.

The two NIAS Chinese breeds have been kept as conservation flocks in Vietnam. It is clear from this study that conservation effort should not pay attention to conserve the two breeds as a part of Vietnamese chicken gene pool as discussed in Chapter 5 but these two NIAS Chinese breeds could be kept as a reservoir for breeding purposes to improve performance of local breeds.

In a formal decision making process, it is necessary to define what are the goals of conservation. This thesis defines conservation priorities to identify which Vietnamese chicken breeds should be conserved and how to spend given limited funds to maximize genetic diversity maintained between breeds and to reduce their extinction probabilities. The current national conservation programs is based on pre-define breeds and this is a limitation especially for the Northern Vietnamese chicken breeds which make up one unstructured gene pool as indicated in Chapter 2. This study should help decision-makers with a better understanding of the consequences of alternative investment strategies for conservation. These strategies could be directly applied in the national conservation programs for other Vietnamese indigenous animal species, such as pig, cattle, etc. if the objective to conserve these species is the same as in this study.

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